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Gravimetric study of Phlegrean Fields Caldera: Mount of Cuma – Archiaverno area

M. Giovanna Fiume
Dipartimento di Geofisica e Vulcanologia, Università degli Studi «Federico II», L.go S. Marcellino 10, Napoli, Italy

Key words: Gravity, Phlegrean Fields, Caldera.

Abstract. Purpose of this paper is the geophysical characterization of the underground structure of a particular area of the volcanic Phlegrean district: Mount of Cuma and the adjacent area of Archiaverno.

New microgravimetric data, measured along a profile crossing W to E the Phlegrean area and passing nearby the Cuma and the Archiaverno lake, were obtained and interpreted. Additionally, in order to get more information, the latest aeromagnetic data of these areas were digitized. All the information arising from this kind of study allowed the structural knowledge of a very complex area to be increased. In fact, the hypotheses about the dimension and the event leading to the formation of the calderic structure connected to the Phlegrean Fields volcanic district, are still various and debated.

Riassunto. Studio Gravimetrico della Caldera dei Campi Flegrei: area del monte Cuma-Archiaverno.

Nell'area dei Campi Flegrei sono stati acquisiti nuovi dati microgravimetrici di dettaglio lungo un profilo che parte dall'area di M. di Cuma ed arriva fino a Pozzuoli. Dopo la fase di correzione ed elaborazione dei dati rilevati, sono state analizzate ed interpretate, da un punto di vista strutturale, le aree M. di Cuma e dell'Archiaverno caratterizzate dalla presenza di due anomalie gravimetriche positive. Il modello scaturito da tale interpretazione ipotizza la presenza di un doppio ribassamento strutturale al di sotto delle aree in questione.

Introduction

The Phlegrean area is located inside the Piana Campana graben, west of Naples and is roughly 70 square kilometers wide (Fig. 1). This depressed structure formed on the western edge of the Appenninic chain since the Upper Pliocene, probably in association with the opening of the Tyrrhenian sea. The Phlegrean Fields volcanic complex, together with Ischia and

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Procida, represents a complicated volcanic system formed by a mainly pyroclastic monogenic group of small volcanoes lined up in an east-west direction. They fed an activity characterized by potassic chemism (Di Girolamo *et al.*, 1984).

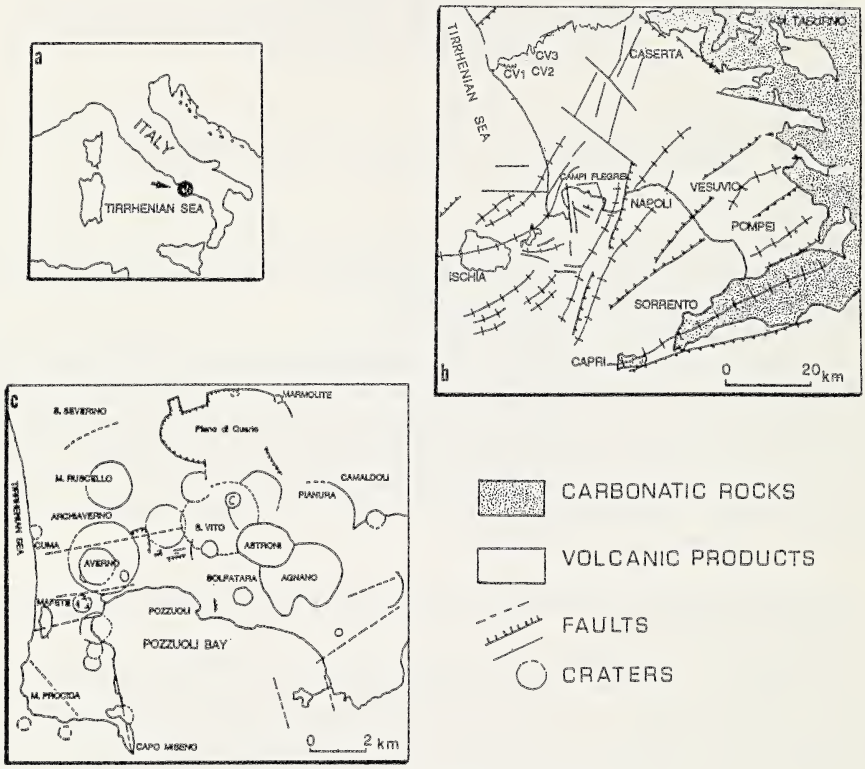

FIGURE 1 – Location of Campi Flegrei

The majority of the present products are pyroclastic in nature; lava flows and domes are very scanty. The distribution of the ejected volumes in relation to the age, indicates that the activity has migrated towards the inner part of the caldera, characterized by decreasing parossistic phases. The explosive Phlegrean activity is characterized by water-magma interaction. Events without hydromagmatic components are extremely rare. The most common products are alcaline trachytes which represent the final product of the low content potassium series. They are the products of recrystallization processes which happened in a relatively small-depth environment.

Description of Cuma-Archiaverno area

In the Mount of Cuma area a small isolated hill is present, which emerges from the plain between the sea and the occidental hills of the Archiaverno-Averno system (Fig. 2). The hill is made of a partially outcropping trachytic lavic dome (>0.01 m.y.) covered either by old products or by more recent products. Such a dome is intensely fractured, even if particularly important faults or fractures could not be pointed out. This kind of stratigraphic sequence is also found in other areas located at the edges of the volcanic Phlegrean district.

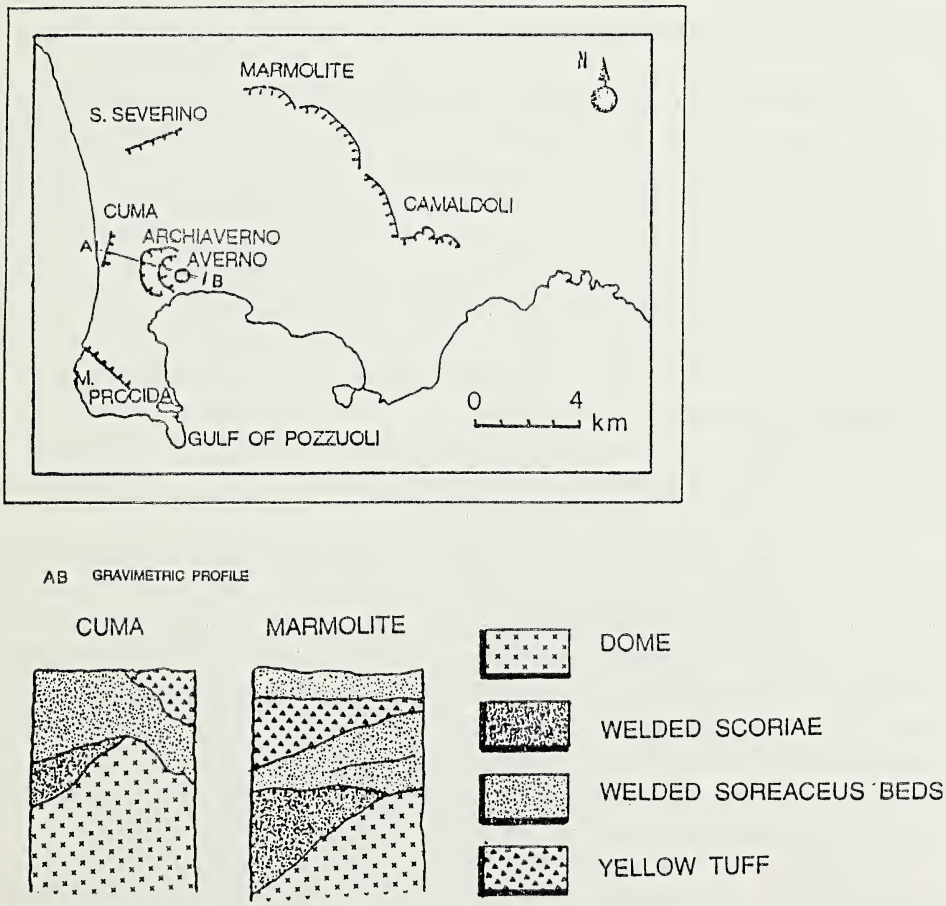

Figure 2 – Location of stratigraphic section

Fig. 2 shows the stratigraphic section of Mount of Cuma and of another fringe area which more or less have the same stratigraphyc and structural characteristic (Marmolite). East of this structure there is a younger feature (Archiaverno) (0.01 m. y.) made of yellow tuff. It is made up of an almost concentric rise at the Averno. This rise was interpreted by De Lorenzo (1904) as the evidence of a crater bigger than the Averno crater. The rest of these apparatus is represented by the mount of Ginestra and the northern portion of the Averno lake. The remaining portion was probably reduced by tettonic collapses and was morphologically modified on the south-west and south-east sides by the Baia, Fondi di Baia and Mount Nuovo volcanoes.

The Phlegrean Fields Caldera

The presence of a collapsed structure connected to the Phlegrean Fields is known either by geological and volcanological observation at the surface and by the analysis of geophysical data. Many and sometimes contrasting theories exist about the limits of this collapse and the generating event. About the first hypothesis we recall Rittmann (1950) which hypothesizes the formation of the Phlegrean caldera as due to the parossistic eruption of the Campanian grey tuff which would have caused the collapse of the presumed stratovulcano called Archiflegreo. Later, Di Girolamo (1970); Barberi *et al.* (1978) supposed that the formation of the Campanian Ignimbrite (Campanian grey tuff) was due to a series of eruptions happening along the fractures located north of the Phlegrean Fields. These eruptions caused the collapse of a vast area of the Phlegrean Fields and a portion of the bay of Naples.

Rosi *et al.* (1983); Rosi and Sbrana (1987), partially accepting the Rittman's hypotesis, recognized the line formed by M. of Procida, Cuma, S. Severino, Marmolite, Camaldoli as the edge of the Phlegrean caldera, caused by the collapse of a magmatic chamber after the eruption of the Campanian Ignimbrite. It must be said that clear geological signs which may confirm the existence of a "step" due to the caldera edge are not recognizable, at least not morphologically. Finally, based on the latest studies, Lirer *et al.* (1987) hypothesized that the formation of the Phlegrean caldera was due to another big eruption (Neapolitan Yellow Tuff).

According to this study, after the emplacement of the yellow tuff, a collapse of the central part of the Phlegrean Fields arised. The yellow tuff remained only on the edges because there they were supported by older

volcanic structures. Such a collapse would be limited by faults having Appenninic and Antiappenninic direction, which in turn would become the place for other new activity (Capo Miseno-Porto Miseno-Bacoli-Punta Epitaffio-Archiaverno). This hypothesis was recently revaluated by Scandone *et al.* (1991), Fedi *et al.* (1991). They, interpreted the caldera collapse as less extended than the previous hypothesis (Rittmann, 1950; Rosi & Sbrana, 1987) and considered M. of Procida, Cuma, S. Severino, Marmolite, Camaldoli areas, not as the caldera edge, but as completely outside the caldera.

Geographical data

As previously mentioned, the presence of a large gravity low (Fig. 3), the interpretation of the other geophysical data and the drilling data (Cassano & La Torre, 1987; Fedi *et al.*, 1991) make probable a collapse in the Pozzuoli bay.

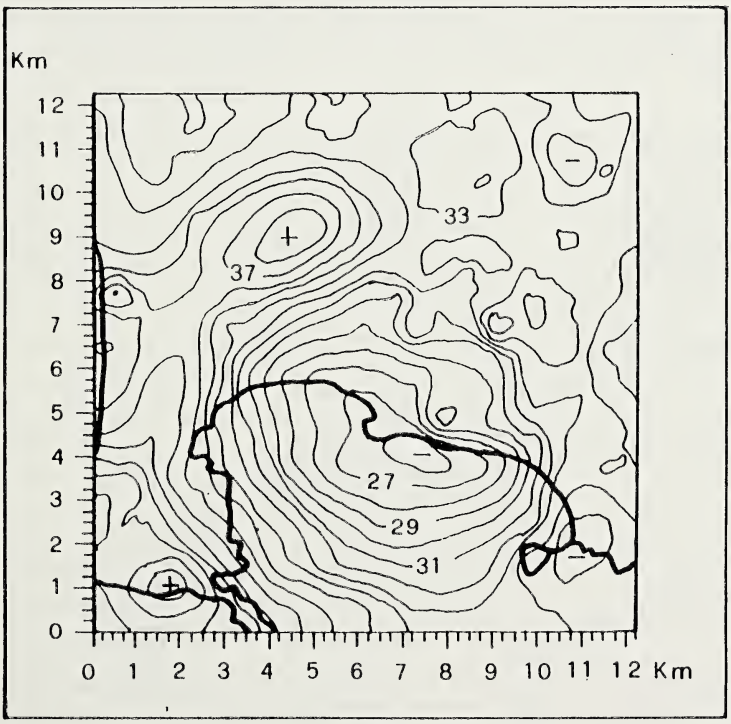

Figure 3 – Campi Flegrei gravity anomaly (Agip, 1987). Contour intervals 1 mGal

The analysis of the samples taken from this drillings, points out intense phenomena of compactation and hydrothermal reactions which, due to the increasing of the tuff and tufites density, makes the gravimetric interpretation difficult. As a matter of fact, these phenomena strongly decreased the density contrast among the various lithotypes. All this points out a very complicated situation and the difficulty to draw a density model, either because of the several, rapid succession of events and because of the high temperature. Furthermore, the effects related to the high temperature on the volcanic rocks are particularly supported by the analysis of magnetic data (Nunziata & Rapolla 1981; Agip, 1987). This because of phenomena of demagnetization due to recent or past circulation of hot waters. Such a phenomenon, pointed out in the M. of Cuma-Archiaverno areas too, causes a very scant correlation between magnetic and gravimetric anomalies (Fig. 4).

Gravimetric study of the Phlegrean Caldera

Gravimetric data acquisition

New detailed microgravimetric data along profiles were collected in the studied area (Fiume, 1991) in order to study the medium to shallow

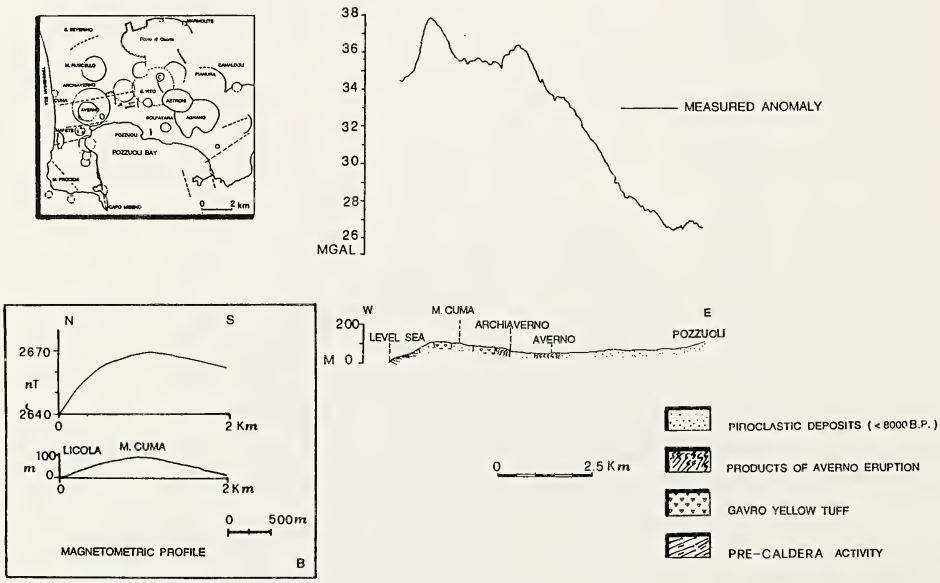

Figure 4 – Gravimetric profile (new data) and magnetometric profile (Agip, 1970)

depth structures. A D-type La Coste microgravimeter was utilized during the field study phase. Distances between stations were 50 m. on overage. Gravity values were referred to the Capodimonte main gravity value (Cannizzo *et al.*, 1978). In particular, in order to calculate the Bouguer anomaly, a density value of 1.4 g/cmc was utilized according to the data of the shallow wells existing in the area (Ducci & Rippa, 1988). The analized profile, shown on Fig. 4a, begins from Mount of Cuma-Archiaverno area and ends at Pozzuoli area, crossing the caldera in an east to west direction; along this profile it is possible to recognize a negative gravity trend of the intermediate frequency band which starts from the Averno area and reaches the minimum values to wards the Pozzuoli bay. Various filterings were performed, in order to separate the deep contributes from the more shallow ones. A cut-off wavelength of 5 km was chosen to separate the caldera contributes from the shallover ones (Fig. 5).

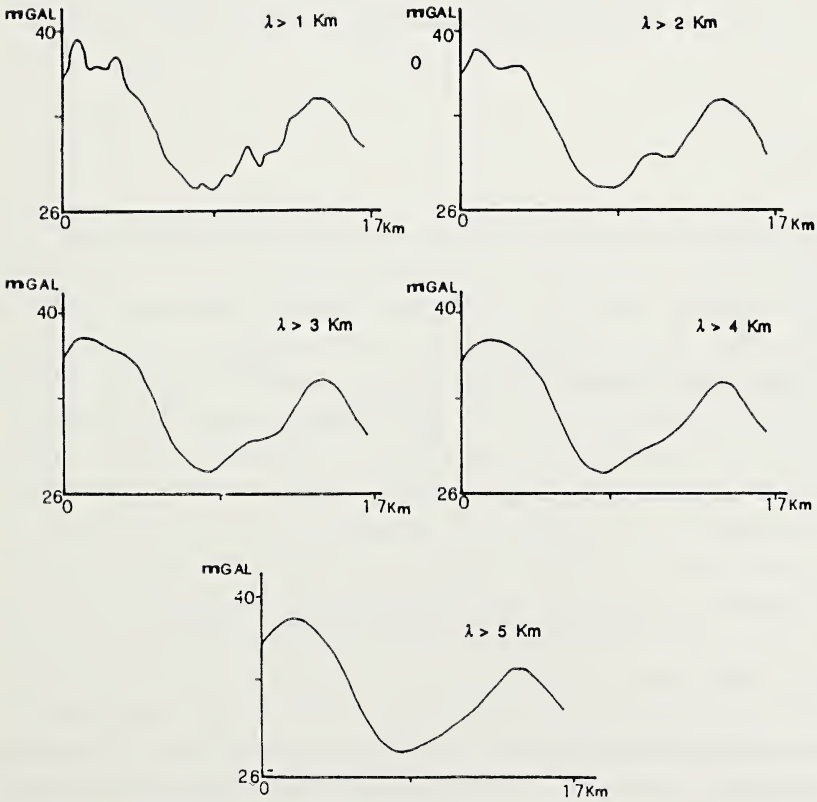

FIGURE 5 – Gravimetric filtered profile (M. Cuma-Fuorigrotta)

Interpretation of the data

A quantitative analysis was performed on the above mentioned residual profile in the Mount of Cuma-Archiaverno area. As can be seen on Fig. 4 a these two areas, which are chronologically and volcanologically different, are characterized by the presence of two gravimetric highs having slightly different amplitudes. Given the presence of an outcrop (from 5m up to 40 m. to sea-level) of the trachytic structure, was hypotesized a simple two layer density model constrained on the surface by the outcropping points of the trachytic structure (Mount of Cuma). The utilized density for the trachytic sequence is 2.5 g/cmc. The density attributed to the upper pyroclastics was 1.8 g/cmc. The suggested quantitative model, obtained by a 2D inversion program is reported on Fig. 6. The Agip aeromagnetic data of the Mount of Cuma area were digitized too, along a nort-south profile (Agip, 1987), in order to attempt a correlation between the two sets of geophysical data, as reported on Fig. 4b.

Results

As already stated above, mount of Cuma area, together with S. Severino, Marmolite, Camaldoli, M. of Procida represent in some Authors' opinion a border area of the Phlegrean caldera (Rittmann, 1950; Rosi & Sbrana, 1987; hypothesis A). More recent studies on the contrary, (Di Girolamo *et al.* 1984; Lirer *et al.* 1987; Scandone *et al.* 1991) hypothesize the above mentioned areas to be completely uninvolved zones in the collapse (hypothesis B). The quantitative model shown on Fig. 6, points out the presence of a high density shallow structure, lowering toward the Averno lake area. A kind of double depression results in this area between M. of Cuma and the Archiaverno and between this structure and Averno lake. Regarding the densities utilized in the structural model, the 2.5 g/cmc value of the dense layer could be everywhere representative of one kind of lava. As said, this is contrained only close to M. of Cuma where, it is possible to recognize the trachytic mass in the outcrop. Regarding the rest of the model, the utilized density could be associated even with compacted and alterated pyroclastic materials due to old or recent circulation of underground waters.

From a structural point of view, the limit of the first depression coincides at the surface with the inner edge of Mount of Cuma. In this kind of structure it is possibile to recognize the hypothesis A. The hypothesis B instead associates the formation of the caldera with the Neapolitan Yellow Tuff eruption. In this case the limits of the collapse in question would be more internal, and in particular recognizable with the Capo Miseno-Baco-li-Punta Epitaffio-Archiaverno volcanic line. The model proposed in the study would even explain the second depression close to the Archiaverno-Averno lake, as shown on Fig. 6. Briefly, this structural model (Fig. 6) could

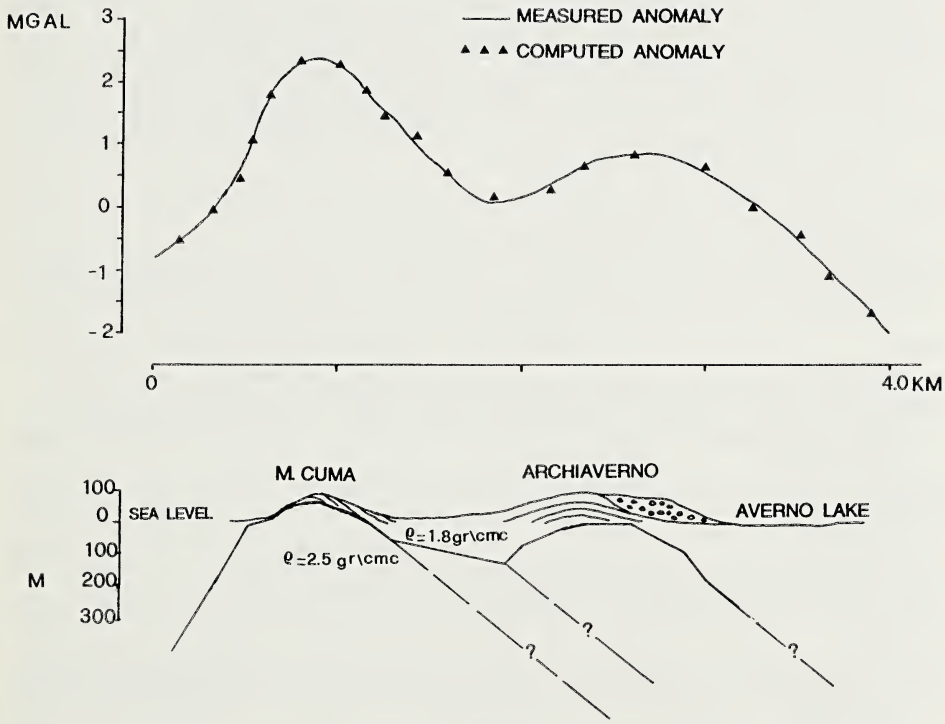

FIGURE 6 – Model of M. Cuma-Archiaverno

be justified by the succession of the two above stated eruptions. These eruptions in turn caused the emptyng of the magmatic chamber which would be the cause of the formation of the two calderic collapses.

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First report on the fauna of Tammaro valley

Buonomo P.[1], Mirabella P.[2], de Filippo G.[1], Milone M.[2]

[1] Studi di Ecologia Applicata, via Caravaggio 143Y, 80126, Napoli, Italy

[2] Dipartimento di Zoologia, Università degli Studi «Federico II» di Napoli, via Mezzocannone 8, 80126 Napoli, Italy

Key words: Mammals, birds, macrobenthos, butterflies, Tammaro river.

Summary. Faunistic researches have been carried out along Tammaro river from 1984 to 1991, in relation to a wider programme for the environmental monitoring. Mammals, Birds, fresh water Macrobenthofauna and Lepidoptera Rhopalocera have been studied and the first results are here reported. We have found 12 species of Mammals and 66 of Birds (18 are resident and breeding, 39 only breeding, 47 migrant, 32 wintering, 3 non breeding summer visitors). As regards the Macrobenthofauna we have found 5 genera of Plecoptera, 6 families of Trichoptera, 11 genera of Ephemeroptera, 5 families of Coleoptera, 6 genera of Odonata, 5 families of Diptera, 2 genera of Heteroptera, 1 family and 1 order of Crustacea, 2 genera of Gasteropoda, 3 families of Oligochaeta, 1 taxon of Acarina. Among Lepidoptera we have identified 13 species of Rhopalocera during the autumn. At a first analysis of data a good species diversity results in the study area, but natural habitats are continuously decreasing.

Riassunto. **Primo contributo alla conoscenza della valle del Tammaro.**
Le ricerche faunistiche nella valle del Tammaro (Sannio), svolte nel periodo 1984-1991, sono inquadrate nell'analisi della fauna appenninica in aree a rischio a causa di rilevanti attività antropiche come la costruzione di invasi. In questo resoconto vengono presentate le prime liste faunistiche delle specie di Mammiferi, Uccelli, Lepidotteri Ropaloceri e dei taxa riscontrabili operando un'analisi del macrobenthos. Sono state riscontrate 12 specie di Mammiferi, 66 specie di Uccelli, di cui 18 residenti, 39 solo nidificanti, 47 migranti, 32 svernanti, 3 estivanti. Tra il macrobenthos ritroviamo i seguenti taxa: cinque generi di Plecotteri, sei famiglie di Tricotteri, 11 generi di Efemerotteri, cinque famiglie di Coleotteri, sei generi di Odonati, cinque famiglie di Ditteri, due generi di Eterotteri, una famiglia e un genere di Crostacei, due generi di Gasteropodi, tre famiglie di Oligocheti, un gruppo di Acari. Infine sono state identificate 13 specie di Lepidotteri Ropaloceri relative al solo periodo autunnale. In particolare tra l'avifauna e la bentofauna si osserva una buona diversità di specie con alcune emergenze faunistiche. Per cui, anche se, con la notevole mosaicizzazione del territorio, si osserva una probabile diminuzione degli habitat naturali, l'area del Tammaro mostra un discreto interesse faunistico e probabilmente una buona qualità ambientale.

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Introduction

The Tammaro river rises in the Sannio's Appennines through a series of springs in the Sepino commune (CB). It flows into the Calore river near the railway station of Paduli (BN). Its drainage-basin (673 sq.km) develops from NW to SE between the Matese massif and the Sannio mountains downstream the Sella of Vinchiaturo (Carta della Montagna, 1976; Plensio, 1978). The river crosses through a series of hills characterized by miocene soils and more recent pliocene sediments. A sufficient stability occurs along the slopes where prevalent arenaceous and/or carbonate soils outcrop; whereas in the land characterized by clayey soils it is possible to observe a considerable geomorphologic trouble, independently by the slope. The territory was inhabited since the prehistory, but it owes his development to the seasonal activity linked to the moving of the herds since the Roman Age. The basin develops along the cattle-track Pescasseroli-Candela (AAVV, 1990). The man-made landscape is typical of the Appennines and it is also defined by the stock-raising, by the farming, by the little towns developed along the historical lines of communication. On the landscape fragmentariness, expanded until now, are superimposed recent phenomena of little industrialization, intensive tobacco growing and river sand mines.

Since the half of the 80's a dam is under construction near Ponte Ligustino, in order to utilize the Tammaro-water to irrigate the fields.

Fauna is practically unknown along the valley if we consider the few and fragmentary informations got from little local issues, without a scientific purpose, (Plensio, 1978) and those ones, however incomplete, got from the "Carta Faunistica della Campania" (Grassi & Milone, 1985) and from the Campania Breeding Birds Atlas (Fraissinet & Kalby, 1987).

In this picture the Department of Zoology has started a research programme over all the areas of Campania where there are dams under construction or about to be planned with three principal aims: 1. to investigate the Fauna of the Appennines in the unknown areas, 2. to preserve the faunistic emergencies and 3. to evaluate and monitor the impact of a dam on the biological communities.

This report is the first contribution to the fauna analysis of the Tammaro valley's.

Study area

The considered area (fig. 1) is located between the "Varco di Vinchiaturo" (CB) (A) and the place-name "Monteleone" (B) near Pescosannita (BN); its altitude varies from 590 m (from sea-level), "Fontana della Tenda" (C), to 284 m, "Monteleone" (B).

The Tammaro is a torrent-like river, but it often shows wide bends. The river-bed is prevalently in its natural state except where the roads or the railways cross it, in these cases the embankments are reinforced with building materials. The banks are covered with the vegetation that, somewhere, in some spots, takes up very small space (only 1 meter). The riparian bush is constituted by willow-, elm-, ash-, alder- tree, tamarisk, hornbeam. In the meandering-areas we can see *Carex* and *Typha*. Near "Ponte Pescosardo" (D) and near "Ponte Ligustino" (E), since 1987 , owing to the construction of the dam, the area is characterized by marshes because of the excavations and of the continual stream deviation.

Methods

Data relating to this work have been collected from 1984 to 1991: Mammals, Birds, Macrobenthofauna and Butterflies were studied.

The researches into the Mammals are connected to the Project "Mammals of Italy Atlas", started in 1990. They were carried out through direct observations and by examining the tracks found along the banks and the river-bed. The direct observations refer to Mammals occasionally detected during the other surveys or to animals found died. The indirect observations regard tracks or spraints. Through the analysis of carnivorous' faeces (Prigioni, 1991) it is possible to show the presence of Insectivora and Rodentia. The determination of the species is possible through the analysis of bones (Toschi & Lanza, 1959; Toschi, 1965) and coats (Debroit *et al.*, 1982).

Birds have been censused by the Emlen transect method (1971). The transects, 1 kilometre long, were carried out early in the morning, 1 hour after the sunrise, in particular during the breeding (April-July) and the wintering (December-February) periods. The census was taken year after year in the 1985-91 period in the following localities: "Fontana della Tenda (C), Ponte Principe (F), Ponte Stretto (G), Ponte Pescosardo (D), Ponte Ligustino (E), Monteleone (B)" .

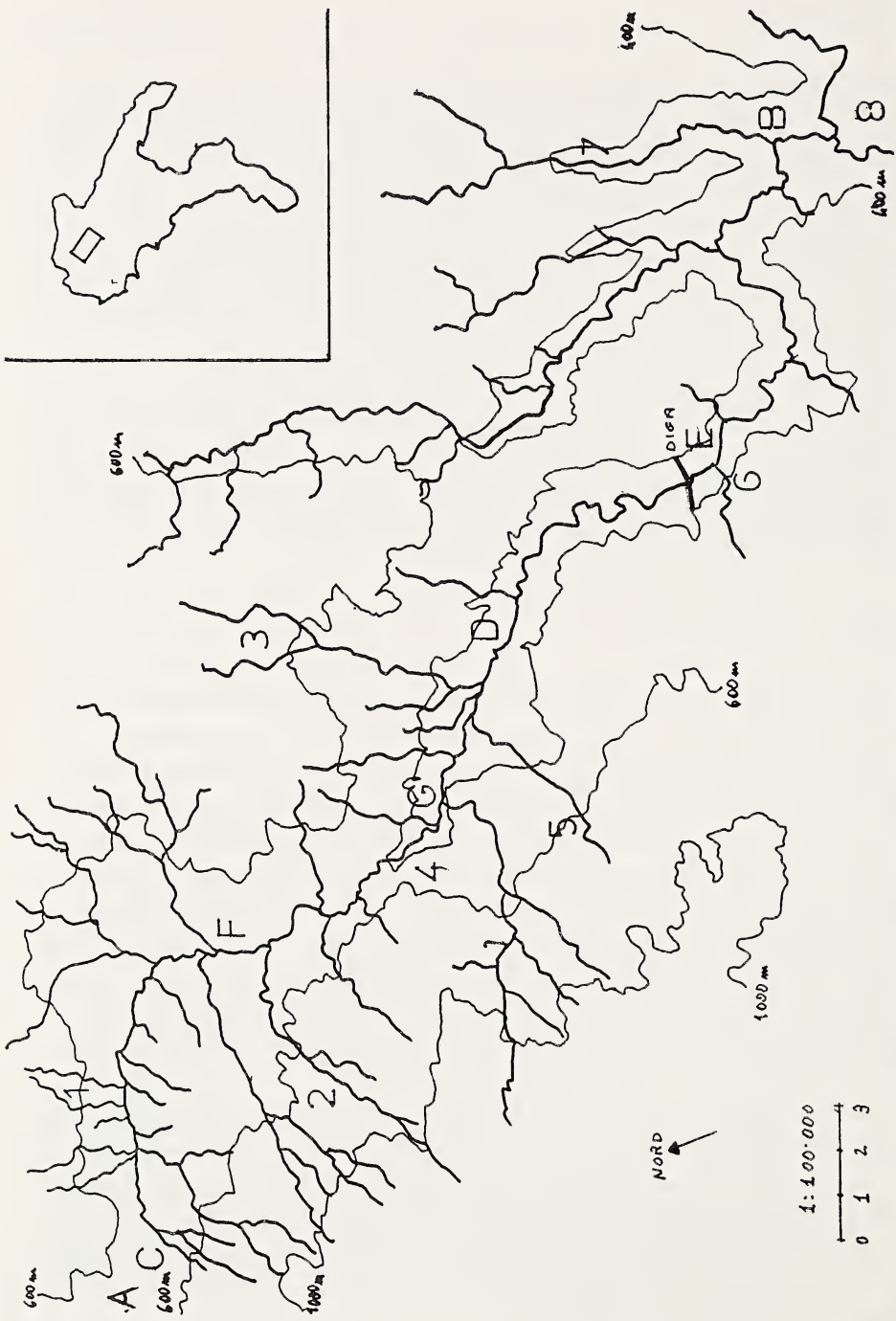

FIGURE 1 – Location of Tammaro valley. Localities – A: Varco di Vinchiaturo; B: Monteleone; C: Fontana della Tenda; D: Ponte Pescosardo; E: Ponte Ligustino; F: Ponte Principe; G: Ponte Stretto; *Main towns:* 1: S. Giuliano del Sannio; 2: Sepino; 3: S. Croce del Sannio; 4: Sassinoro; 5: Morcone; 6: Campolattaro; 7: Reino; 8: Pesco Sannita.

The Macrobenthofauna was collected in the same localities, during the 1990-91 years, according to Ghetti (1986). The samples were collected in spring and autumn by utilizing the "surber" according to the tipology of the river' stretch; the samples were determined in the laboratory until a taxa's level useful and necessary to obtain the values of the environment's quality (E.B.I.: Ghetti, modified, 1986; I.B.G.: by Verneaux, 1983).

The Lepidoptera (Rhopalocera) were collected covering linear transects, along the river. Until now only the autumnal period (1991) it has been investigated. The species' determination was carried out according to Higgins & Riley (1983).

Results

In table I the 12 species of Mammals identified along the banks and the river-bed are indicated.

Table I – The Mammals of the Tammaro banks

Species
Erinaceus europeus
Talpa europea
Lepus capensis
Myotis glis
Arvicola terrestris
Rattus norvegicus
Mus musculus
Vulpes vulpes
Mustela nivalis
Martes foina
M. martes
Sus scrofa

The Avifauna identified during the transect counts is shown in table II; it is also described the phenology of the species. 66 species have been identified: 18 are considered as sedentary-, 39 as breeding- (in addition to the sedentary species), 47 as migratory-, 32 as wintering-, 3 as non breeding summer visitor-species.

TABLE II – Chek-list of the birds of the Tammaro banks with their phenological status. B=breeding; S=sedentary; M=migratory; W=wintering; E=non breeding summer visitor.

Species	Phenology
Ardea cinerea	M,W,E
Buteo buteo	M,W,E
Milvus milvus	B,M,W,E
M. migrans	M,W
Falco tinnunculus	S
Gallinula chloropus	S
Charadrius dubius	B,M,W
Scolopax rusticola	B,M,W
Vanellus vanellus	M,W
Tringa ochropus	M,W
Columba livia	S
C. palumbus	B,M,W
Streptopelia turtur	B,M
Apus apus	B,M
Alcedo atthis	B,M,W
Cuculus canorus	S
Jynx torquilla	B,M,W
Lullula arborea	B,M
Galerida cristata	B,M
Alauda arvensis	B,M,W
Hirundo rustica	B,M
Delichon urbica	B,M
Anthus campestris	B,M
A. pratensis	M,W
Motacilla cinerea	B,M
M. alba	B,M,W
Troglodytes troglodytes	S
Prunella modularis	M,W
Erithacus rubecula	B,M,W
Luscinia megarhynchos	B,M
Phoenicurus ochrurus	M,W
Saxicola torquata	B,M,W

cont. TABLE II

Species	Phenology
Oenanthe oenanthe	B,M
Turdus merula	S
T. philomelos	M,W
Acrocephalus schoenobaenus	B,M,W
A. scirpaceus	B,M
A. arundinaceus	B,M
Cettia cettii	B,M,W
Cisticola juncidis	S
Sylvia melanocephala	S
S. communis	B,M
S. atricapilla	B,M,W
Phylloscopus collybita	B,M,W
Muscicapa striata	B,M
Aegithalos caudatus	B,M,W
Parus caeruleus	S
P. major	S
Remiz pendolinus	S
Sitta europea	S
Lanius collurio	B,M
Garrulus glandarius	S
Pica pica	S
Corvus corone cornix	S
Passer italiae	S
P. montanus	S
Fringilla coelebs	B,M,W
Serinus serinus	B,M,W
Carduelis chloris	B,M,W
C. carduelis	B,M,W
C. spinus	B,M,W
C. cannabina	B,M,W
Emberiza citrinella	B,M
E. cia	B,W
E. cirlus	B,M,W
Miliaria calandra	S

The taxa of the Macrobenthofauna are listed according to the systematic level required to determinate the water's quality. The specimens identified belong to 5 genera of Plecoptera, 6 families of Trichoptera, 11 genera of Ephemeroptera, 5 families of Coleoptera, 6 genera of Odonata, 5 families of Diptera, 2 genera of Heteroptera, 1 family and 1 order of Crustacea, 2 genera of Gasteropoda, 3 families of Oligochaeta, 1 taxon of Acarina (table III).

13 Butterflies species until now are identified (table IV).

TABLE III – Macrobenthofaunistic taxa of Tammaro river.

Order or class	Family	Genus
Plecoptera	Capniidae	*Capnia*
	Nemauridae	*Protonemura*
	Perlodidae	*Isoperla*
		Perlodes
	Taeniopterygidae	*Brachyptera*
Trichoptera	Limnephilidae	
	Psychomyidae	
	Rhyacophilidae	
	Polycentropodidae	
	Hydropsychidae	
	Philopotamidae	
Ephemeroptera	Baetidae	*Baetis*
		Pseudocentroptilum
Ephemerellidae	*Ephemerella*	
	Leptophlebiidae	*Paraleptophlebia*
		Habrophlebia
	Heptageniidae	*Ecdyonurus*
		Rhithrogena
		Epeorus
		Electrogena
	Caenidae	*Caenis*
	Oligoneuridae	*Oligoneuriella*
Coleoptera	Elminthidae	
	Hydrophilidae	
	Haliplidae	
	Hydraenidae	
	Dytiscidae	

cont. TABLE III

Order or class	Family	Genus
Odonata Zyg.	Platycnemididae	*Plactynemis*
	Coenagrionidae	*Pyrrhosoma*
	Calopterydidae	*Calopterix*
	Anis. Gomphidae	*Onychogomphus*
	Libellulidae	*Orthetrum*
		Cracothemis
Diptera	Simuliidae	
	Chironomidae	
	Limoniidae	
	Ceratopogonidae	
	Tipulidae	
Heteroptera	Corixidae	*Micronecta*
	Pleidae	*Plea*
Amphipoda	Gammmaridae	
	Cladocera	
Basommatophora	Planorbidae	*Gyraulus*
	Physidae	*Physa*
Tubificida	Tubificidae	
	Enchytraeidae	
Haplotaxida	Lumbricidae	
Hydracarina		

DISCUSSION

In the area of the Tammaro river, the development of the naturalistic research has made possible only a comparison between our data on the Mammals and the Birds and those ones of the "Carta Faunistica della Campania" (Grassi & Milone, 1985) and of the Breeding Birds of Campania Atlas (Fraissinet & Kalby, 1987). This comparison is limited because of the different methods of investigation: the data from the "Carta Faunistica" were reported according with the Commune boundaries and those ones from Atlas were referred to squares 1:25.000 according with I.G.M. maps.

13 Mammals species are reported for the Tammaro area in the "Carta Faunistica". Here we report 12 species: among them lack *Elyomis querci*

TABLE IV – Lepidoptera of the Tammaro banks

Species	Family
Papilio machaon	Papilionidae
Artogeia rapae	Pieridae
Artogeia mannii	
Artogeia napi	
Colias crocea	
Lycaena p. phleas	Lycaenidae
Heodes tityrus	
Everes alcetas	
Polyommatus icarus	
Melitaea didyma	Nymphalidae
Maniola jurtina	Satyridae
Coenonympha pamphilus	
Ochlodes venatus	Hesperiidae

nus, *Muscardinus avellanarius*, *Canis lupus*, *Mustela putorius*, *Meles meles*, *Felis silvestris*, which are reported in the "Carta Faunistica". This difference in the number of species is due to the fact that in the "Carta Faunistica" only the species of agricultural and hunting interest are documented. The only faeces found in the study area are those of *Vulpes vulpes*, but we haven't found bones or coats of micromammals there. As regards the other species we think that it is possible the presence of *Canis lupus* (checked for the Matese massif and the Sannio's mountains), but it would be a very occasional occurrence which is related to the wide mobility of the species along the Appennines (Boitani L., pers. comm.). Not scientific literature report the presence of *Meles meles*, *Mustela putorius* and *Sciurus vulgaris* (Plensio, 1978); we have no evidence for the first two species, and we are sure that *Sciurus vulgaris* is mistaken for *Myotis glis*.

Differing from the "Carta Faunistica" and the Breeding Birds Atlas, our data don't include the night-birds of prey: this is due to the transect method. Other species (as the migratory Charadriiformes, some Ardeidae or Anatidae) are not reported in our check-list may be because of their accidental presence in the study area due to the variability of the climate and of the habitats' typology. A better analysis on the breeding birds is possible by comparing the data from the Atlas with our list (see table II). In the Tammaro basin 60 breeding birds are reported in the Atlas while 57 species are censused in this paper. Species like *Falco subbuteo*, *Coturnix*

coturnix, Phasianus colchicus, Saxicola rubetra, Phylloscopus sibilatrix, Parus palustris, Corvus monedula, Corvus corax, Petronia petronia, select for habitats different from those ones found along the Tammaro river. For this reason they are reported in the Tammaro region by the Atlas, but are not reported in our list. Other species like *Picus viridis, Picoides major, Acrocephalus melanopogon, Hippolais polyglotta, Sylvia cantillans, Certhia brachydactyla, Oriolus oriolus* could be extremely localized on the territory (so that it would be unlikely to take a census of them with the transects). An other hypothesis could be the reduction of suitable breeding habitats in the last years. On the other hand the transect method has proved the breeding of *Milvus milvus, Anthus campestris, Acrocephalus schoenobaenus, A. arundinaceus, Cisticola juncidis, Sitta europea, Remiz pendolinus, Carduelis spinus* which are not inserted in the Atlas. Other species, regarded as "probable breeding" in the Atlas, as *Alcedo atthis, A. scirpaceus, Muscicapa striata, and Corvus corone cornix*, were proved to be surely breeding. It is important the breeding of *Anthus campestris*, which is indicated as a rare breeding species in Campania (de Filippo *et al.*, 1990). Moreover it is very important the breeding of *Acrocephalus schoenobaenus* and *Carduelis spinus* (Conti P., pers. comm.), since these nidifications are the first ones in the Campania District. The ornithological informations described in the work of Plensio (1978) are difficult to interpret: there are species easy to identify like *Perdix perdix* or *Oriolus oriolus*, some hard to identify like *Hippolais polyglotta* or *Regulus regulus*, some typical of other biogeogrhaphic areas like *Parus cristatus*, or some considered the same species such as *Anthus campestris* and *Melanocorypha calandra*.

In this first approach to the benthofaunistic taxa our list of familia and genera can lead to same general considerations. The identification of many genera of Plecoptera shows the presence of fresh-waters rich in oxygen and a stony river-bed, even if the genus *Isoperla* is able enough to endure a light defilement. In the order of Trichoptera we can note the presence of Hydropsychidae where the river is rich in organic substances and the current is moderate; other families like Philopotamidae and Rhyacophilidae are typical of torrent-like rivers. In the order of Ephemeroptera it is important the identification of one species of genus *Caenis* recently observed in the South of Italy (Belfiore C., pers. comm.). Other genera like *Baetis* and *Ephemerella* endure pollution very well, others like *Oligoneuriella* are characteristic of torrents or streams with speedy waters. The Coleoptera Elmididae prefer stony and pebbly stream-beds; other families like Hydrophilidae and Hydraenidae can live in stagnant-waters too. Coleoptera generally prefer bank- microhabitats with shallow water, lots of

vegetation and organic remains. The families of Ceratopogonidae, Chironomidae and Tipulidae (Diptera) are all of sandy- or oozy-bottom rich in organic material. The genus *Physa* (Gasteropoda) suggests the presence of limpid and oxygenated fresh-water. On the other hand the Oligochaeta prefers rivers with sandy- or oozy-bottoms, aquatic vegetation and organic remains. These observations show a high environmental variability and a considerable lack of information on the Appennines Macrobenthofauna.

The Lepidoptera here identified are generally species with wide range in Italy; their occurrence denotes a great floral diversity caused by a considerable mosaicism in the landscape. Further considerations are not possible because the survey concerns only the late summer and it lacks all the spring species.

Today the Tammaro valley's is not "... the very great forest...very terrible and shadowy for the several high and branched oaks ..." as a geographer Dominican friar wrote in 1681 neither that one described by the historiographer Galanti in 1770 (Plensio, 1978).

A general analysis of our data displays a considerable mosaicism of the area, a good species diversity independently by the decrease of natural habitats but according to the high environmental variability. It is important, now, to carry on the naturalistic research in an unknown area to hypothesize a suitable management through a valuation of the environmental quality.

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Recent faunistic and ecotoxicological arrangement of the terminal tract of the Sarno river (Campania, Italy)

Soppelsa O., Battaglini F. e Battaglini P.
Dipartimento di Zoologia, Università degli Studi «Federico II», Napoli, Italy

Key words: River pollution, benthic fauna, ecotoxicology.

Abstract. This report describes the present faunistic, ecological, and ecotoxicological environment of the central and terminal part of the Sarno river in Campania. Previous studies considered it as a paradigmatic example of river pollution. Six stations located from the central part of the river to its mouth were selected for this study.

Animal taxa include few species which are typical of a polysaprobic environment. This is due to the inflow of the Cavaiola stream, the most important tributary of the Sarno river and main cause of its pollution.

The station located before the Cavaiola stream still maintains acceptable ecological and faunistic conditions, while after the point of confluence with this stream there is a zonation with river population, highly toxic influenced by the waters of this tributary.

Our research showed that the causes of this pollution are the high number of industries operating along the river, the home waste of the highly and densely urbanised neighbouring area and a poor utilisation of substances used in agriculture.

The comparison between current data and the relevant literature shows a general worsening of the ecological and faunistic condition of the river, making its possible recovery even more difficult.

Riassunto. Recente assetto ecologico e faunistico della bassa valle e foce del fiume Sarno (Campania, Italia).

Il Sarno è situato essenzialmente nella fertile pianura dell'agro nocerino-sarnese. L'affluente più grande è il torrente Cavaiola, altre immissioni sono il Canale Fosso Imperàtore e il Canale di S. Antonio Abate. Sfocia dopo circa ventiquattro chilometri nel Golfo di Napoli al confine dei Comuni di Torre Annunziata e Castellammare di Stabia.

In rapporto alla lunghezza del suo corso e per la notevole urbanizzazione e industrializzazione del suo bacino, il Sarno è sottoposto ad un massiccio sfruttamento, che rende la portata più esigua esponendo il fiume agli attacchi inquinanti.

La presente ricerca ha lo scopo di analizzare l'attuale stato ecologico ed ecotossicologico del tratto medio-terminale del Fiume Sarno che è considerato

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come un esempio paradigmatico di inquinamento fluviale. L'analisi ha interessato sei stazioni di campionamento, da prima del Cavaiola fino alla foce, in un periodo di tempo di circa un anno (ott. 90 – giu. 91) per un totale di cinque serie di prelievi.

In ogni stazione sono state effettuate analisi chimico-fisiche dell'acqua e contemporaneamente è stata raccolta la bentofauna.

La presenza contemporanea di ione ammonio e azoto nitroso indica che parte dell'inquinamento del Sarno è di origine organica.

L'acqua è risultata basica per la presenza di NH_3. Tuttavia l'azione basica dell'NH_3 è tamponata da acidi, come l'H_2S, provenienti anch'essi dalla decomposizione di materiali organici o scaricati dalle industrie conserviere; si ottiene così una stabilizzazione del pH su valori leggermente basici. I solfuri presenti provengono anche dagli scarichi di alcuni processi della lavorazione conciaria ove viene usato Na_2S. Nel periodo estivo le condizioni divengono più gravi per la diminuita portata del fiume e per l'elevata temperatura che rende l'ossigeno, già in condizioni normali prossimo agli 0 mg/L, meno solubile in acqua.

Dai risultati ottenuti si deduce che le acque del Sarno sono "molto dure".

Si sono registrati, nel corso dei prelievi, anomali sbalzi della concentrazione dello ione Cl^- a valle del Cavaiola; probabilmente il forte aumento dei cloruri è dovuto alla dissalazione delle pelli.

Dai valori degli indici di dominanza e di diversità si evince che il tratto di fiume compreso tra l'immissione del Cavaiola e la foce risulta il più danneggiato, sebbene si noti un leggero recupero nel tratto terminale. La stazione 5 a monte della confluenza, dove sono presenti tutti e 6 i phyla, conta il maggior numero di specie, sono infatti presenti oltre ad un 33.5% di Nematodi, un 18.6% di Rotiferi e un 16.3% di larve di Ditteri (essenzialmente del genere *Chironomus*), anche un 12% di Copepodi, un 5% di Gasteropodi tra cui i generi *Planorbis e Physa* e un 2.4% di Emitteri. Nella stazione 4 si può notare la differente composizione faunistica tra le due sponde a causa della confluenza del Torrente Cavaiola. Nelle stazioni 3 e 2 sono presenti solo Turbellari (*Stenostomum* sp., *Mesostoma* sp.), Rotiferi (*Rotaria neptunia*), Nematodi, Oligocheti (*Tubifex tubifex*) e Ditteri (anche qui larve di *Chironomus*). Nella stazione 1 sono stati raccolti anche alcuni Copepodi.

Complessivamente sono stati raccolti 2226 animali appartenenti a 6 phyla (Platelminti, Rotiferi, Nematodi, Molluschi, Anellidi, Artropodi) e raggruppabili in 13 taxa.

In definitiva si è notata la presenza di tre zone differenziate tra loro per la qualità e quantità di individui raccolti. La prima zona, identificabile nella stazione 5 è la più ricca di phyla ed ha una struttura faunistica tale da poter essere compresa tra le classi alfa e beta mesosaprobie del sistema saprobio. La seconda zona, la più devastata dall'inquinamento, comprende il tratto subito a valle dell'immissione del Cavaiola ed è definibile come metasaprobia. La terza ed ultima zona è identificabile nelle stazioni 3, 2, e 1 ed ha accentuate caratteristiche polisaprobie.

I dati faunistici, in perfetto accordo con i parametri fisico-chimici, hanno permesso di ricondurre a due tipi fondamentali le cause dell'inquinamento: il primo alla grossa presenza delle industrie, il secondo agli scarichi domestici; causa minore ma non per questo trascurabile è l'inquinamento di origine agricola.

La stazione a monte del Cavaiola conserva ancora condizioni accettabili. Dalla confluenza del Cavaiola in poi si ha una "zonazione", infatti le acque di questo affluente costituiscono una vera e propria barriera ecologica alla diffusione degli

animali; gli indici di dominanza diventano altissimi e quelli di diversità bassi rendendo evidente la condizione di forte stress.

Il confronto dei presenti dati con quelli in letteratura mostra un peggioramento generale delle condizioni del fiume; gli apporti inquinanti non riescono ad essere smaltiti e ciò comporta un allontanamento dalle condizioni di normalità. Altro ostacolo alla formazione di biocenosi ben definite sono le forti oscillazioni stagionali dei reflui.

Introduction

The Sarno river flows in a fertile plain surrounded on the North side by the Vesuvius and on the other sides by the Lattari Mountains. It flows through eight cities. It is originated by three sources: S. Maria della Foce (2.5 m^3/s), used for a water system and a hydroelectric power plant; Acqua di Palazzo (3 m^3/s), located at the town of Sarno; and Acqua di S. Marino (2 m^3/s). The three sources flow together originating a single river: the Sarno river.

The largest tributary is the Cavaiola stream, which increases its flow rate due to the inflow of the Solofrana stream. The Cavaiola stream takes origin at about 1,300 m above sea level from the Garofalo Mountain near Solofra and runs for almost 35 km before flowing into the main stream of the Sarno river at San Marzano. Other tributaries flowing into the Sarno river are the Fosso Imperatore canal and the S. Antonio Abate canal. The Sarno river gives rise to the Bottaro canal at Scafati. This canal was built for the purpose of irrigating the lands of the Pompeii area.

After twenty-four kilometres the Sarno river flows into the Gulf of Naples at the border between the towns of Torre Annunziata and Castellammare di Stabia.

In proportion to its length the Sarno river suffers a heavy agricultural exploitation and the pollution by industrial and domestic wastes, both reducing its flow rate and exposing the river to continuous pollution attacks.

As a matter of fact, the highly urbanized area crossed by the river is characterized by a widespread and potentially polluting vegetable and fruit intensive cultivation and by several preserved food, tanning, paper, and pharmaceutical industries (d'Elia *et al.*, 1974).

In view of the above, it appears clear that the Sarno river is a paradigmatic example of highly altered watercourse. After the faunistic, ecological and general studies (Battaglini, 1979) on the hygienic, sanitary

and pollution aspects (d'Elia, 1988), the ecological data, sensu lato, are no longer present in literature. Due to the ecological, rural and social-economic importance of this river in Campania, it was necessary to examine the current conditions of the central and terminal part of the Sarno river, which is the area most affected by alternative phenomena of anthropic origin.

The location of research stations is very important to verify the sanitary conditions of a river (Battaglini, 1979). In this specific case the location of the stations was easier, as it was based on previous researches (Battaglini *et al.*, 1967 – 1968; d'Elia *et al.*, 1974). However, a survey with relevant sampling-site takings was carried out to detect any changes in the conditions of the river.

Procedures and methods

Five monitoring sites were located along the Sarno river and one along the tributary Cavaiola (see Fig. 1); these are numbered in Arabic numerals from the mouth to the middle of the river.

Station 1 represents the mouth of the river and is situated at about 200 m from the sea.

Station 2 was located beyond the inflow point of the S. Antonio Abate canal and the drain of a paper mill (2.7 km from the sea).

Station 3 is not very far from station 2. It is situated between the towns of Scafati and Pompeii and was selected to obtain a relative control (reference specimen) of the chemical tests for the subsequent stations (1 and 2), as it is located upriver from an important tributary: the canal of S. Antonio Abate (3.825 km from the sea).

Station 4 is located downstream from the inflow point of the Cavaiola stream (10.025 km from the sea), which was analysed by the CAV special station due to its polluting power.

Station 5 is the closest to the source and is located upriver from the confluence with the Cavaiola stream; this station will be considered as a control to all intents and purposes of the research.

The listed stations are located in an area of study that ranges from approximately one kilometre upriver from the inflow of the Cavaiola to its terminal part (see Fig.1), covering a total of 10.350 km.

Samplings were carried out at each station both on the right (R) and on the left bank (L) on the following days 01/10/90, 12/12/90, 26/02/91, 08/05/91 and 26/06/91, proceeding upstream from station 1 to station 5 as

suggested by standard sampling methods for fluvial fauna (Standard Methods, 1989).

One-litre (1 L) water samples were taken and stored in Teflon bottles at each station for the chemical-physical study of the waters. Furthermore, samples of exactly 60 ml were taken for the analysis of oxygen dissolved in the water.

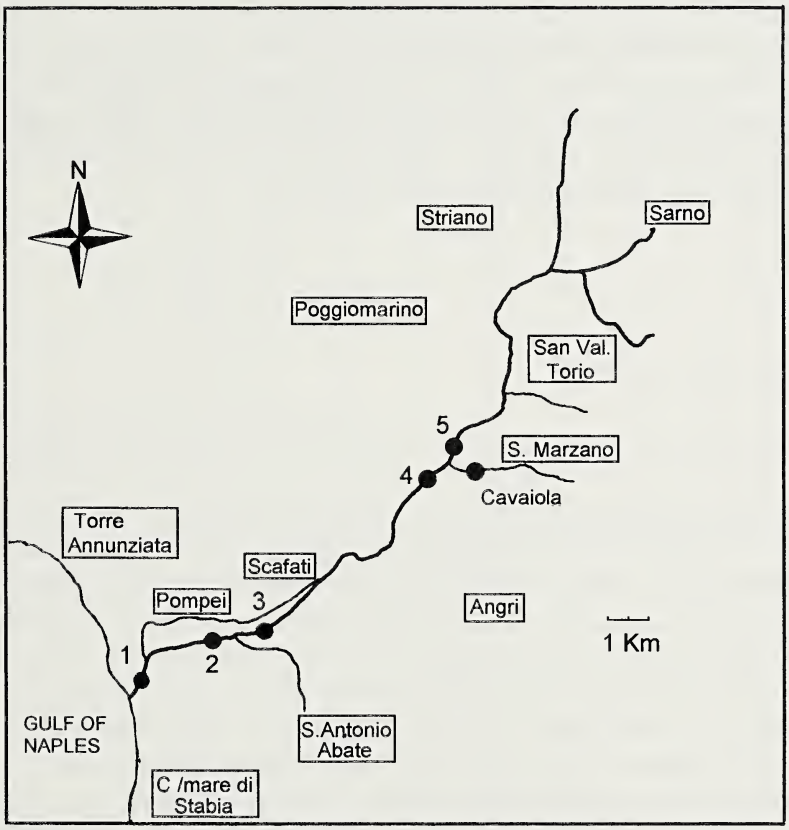

FIGURE 1 – Simplified representation of the Sarno river and of its tributaries; the various sampling stations are reported (●).

The chemical-physical researches concerned the survey of NO^2, NH^{4+}, Cl^-, Fe, Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+},O_2, H_2S, total hardness, alkalinity, temperature (air, water), pH.

Air temperature was measured with a +10 to +40° C scale thermometer, while water temperature was determined by means of a 0 to 50 scale pit thermometer with a 0.5° C accuracy. Spectrophotometry methods were used to analyze the concentration of pollutants.

The concentration of ion magnesium was obtained theoretically subtracting the calcium from total hardness.

The collecting of mesobenthofauna was carried out with a small 30 cm wide and 38 cm deep landing net (approx. 25 L capacity, 0.5 cm mesh diameter). The six samplings were carried out at each station within a time span of 12 h and repeated at intervals of about two months from each other to examine the entire yearly cycle of the river.

The Dominance index of Simpson (Pielou, 1966b), the Difference index of Shannon (Pielou, 1966c), and the similarity index (Pielou, 1966a) were assessed along with the taxonomic determination and the ecological-statistical analysis in order to obtain a more accurate picture of the distribution and the population characteristics.

Results

Chemical-Physical Results

The chemical-physical characteristics of the water taken from both banks of the six stations during the research are reported in detail in Tables 15. The chemical-physical data give an even better idea of the big damage that the Cavaiola stream causes to the Sarno river. As a matter of fact, upriver from this inflow (Station 5) the average O_2 and NH_3 values of the river are 1.25 and 0.33 mg/L, respectively, while at the immediately subsequent station 4L its course is conditioned by the values of the tributary: the average O_2 and NH_3 values are 0.2 and 4.7, respectively.

Thus, it is likely that the Cavaiola stream is responsible both for the increase of organic waste and decrease of the concentration of oxygen dissolved in water. Even if it is obvious that this is due to aerobic-type catabolic processes, it should be noted that catabolic processes are mainly anaerobic due to the very low rate of O_2 and the presence of H_2S. These phenomena are mainly ascribable to the massive number of tanneries operating along the Solofrana stream (a tributary of the Cavaiola stream) and draining waters containing hair and flesh tissues. The great number of unlawful drains flowing into both streams should also be considered.

In addition to the increase of organic substances, the Cavaiola stream is also responsible for the presence of toxic substances such as hexavalent chrome (found through qualitative tests) and for the significant variation of Cl^-, basically due to the hide desalting process (see Tables I-V).

TABLE I – Chemical-physical properties of water samples taken at the various stations on the 1/10/90; L=left bank – R=right bank

1/10/90	Station 1		Station 2		Station 3		Station 4		Station 5		CAV.
	S	D	S	D	S	D	S	D	S	D	
T °C (air)	22	22	27	27	29	29	24	24	24	24	24
T °C (water)	15	14	16	16	16	16	16	16	16	16	16
pH	7.13	7.18	6.95	6.87	7.02	7.00	6.92	7.05	7.06	7.14	7.08
O_2 (mg/L)	–	–	–	–	–	–	–	–	–	–	–
NO_2^- (mg/L)	0.6	0.1	0	0	0.1	0.1	0	2	0.3	2	trace
NH_4^+ (mg/L)	1.1	1.1	1.3	0.7	1.9	0.4	3.5	0.6	0.25	0.5	4.3
Cl^- (mg/L)	75	75	75	75	75	75	300	40	50	50	300
Hardness*	484	488	463	454	445	449	393	430	441	445	392
Ca^{++} (mg/L)	125	126	124	124	122	122	100	122	119	119	95
Mg^{++} (mg/L)	41.2	41.4	36.7	34.5	33.6	34.4	34.4	29.7	34.5	35.4	37
Alcalinity	400	400	465	470	455	455	460	420	432	437	550
CO_2 (mg/L)	30.4	26.8	44.4	54.6	37.0	38.7	47.5	32.0	31.7	27.3	39.2
Fe (total) (mg/L)	0	0	trace	trace	trace	trace	0.6	0	0	0	0.7
Fe^{++} (mg/L)	0	0	0	0	0	0	0	0.1	0	0	0.1
Fe^{+++} (mg/L)	0	0	0	0	0	0	0	0.5	0	0	0.6

* = mg/L $CaCO_3$

TABLE II – Chemical-physical properties of water samples taken at the various stations on the 12/12/90; L=left bank – R=right bank

12/2/90	Station 1		Station 2		Station 3		Station 4		Station 5		CAV.
	S	D	S	D	S	D	S	D	S	D	
T °C (air)	5.0	4.5	8.0	8.0	14.5	14.5	14.0	9.0	9.0	9.0	12.0
T °C (water)	12.5	12.0	13.0	13.0	13.0	13.0	12.0	12.5	12.5	12.5	12.0
pH	7.17	7.15	7.18	7.18	7.25	7.20	7.55	7.33	7.41	7.36	7.39
O_2 (mg/L)	0.4	0.2	0.4	0.2	0.4	0.4	trace	1.6	1.6	1.6	trace
NO_2^- (mg/L)	>2.0	>2.0	>2.0	>2.0	>2.0	2.0	0.1	0.4	0.6	0.6	0.1
NH_4^+ (mg/L)	0.8	1.3	0.8	0.8	1.5	0.6	2.5	0.2	0.3	0.2	3.6
Cl^- (mg/L)	100	100	150	150	150	150	>300	50	45	50	>300
Hardness*	502	484	502	488	472	488	459	454	466	445	516
Ca^{++} (mg/L)	138	135	136	137	132	130	130	129	130	129	138
Mg^{++} (mg/L)	37.7	35.2	38.9	34.8	34.0	39.0	32.2	31.5	33.9	29.4	41.1
Alcalinity	450	470	475	470	450	470	545	425	425	425	>550
CO_2 (mg/L)	25.0	29.0	26.8	26.4	22.0	25.5	12.8	16.7	14.0	15.8	19.0
Fe (total) (mg/L)	0.3	0.2	0.4	0.4	trace	0.3	0.5	0	0	0	0.5
Fe^{++} (mg/L)	0	0	0	0	0	0	0.5	0	0	0	0.5
Fe^{+++} (mg/L)	0.3	0.2	0.4	0.4	trace	0.3	0	0	0	0	0

* = mg/L $CaCO_3$

TABLE III – Chemical-physical properties of water samples taken at the various stations on the 26/02/91; L=left bank – R=right bank

26/2/91	Station 1		Station 2		Station 3		Station 4		Station 5		CAV.
	S	D	S	D	S	D	S	D	S	D	
T °C (air)	7	7	8	8	10	10	11	14	10	14	11
T °C (water)	12.0	12.0	11.5	11.5	12.0	12.0	12.0	12.0	12.0	12.0	12.0
pH	7.19	7.19	7.14	7.19	7.29	7.22	7.74	7.41	7.46	7.42	7.77
O_2 (mg/L)	0.4	0.4	0.6	0.4	0.6	0.6	0.2	0.7	0.8	1.5	0.2
NO_2^- (mg/L)	>2.0	>2.0	1.3	1.3	1.3	1.3	1.3	0.4	0.4	0.4	>2.0
NH_4^+ (mg/L)	1.0	1.0	1.2	1.0	1.2	0.8	2.8	0.4	0.4	0.4	4.5
Cl^- (mg/L)	75	75	75	75	75	75	130	50	50	50	130
Hardness*	509	509	509	509	488	498	310	466	466	473	306
Ca^{++} (mg/L)	140	139	140	140	136	140	95	138	132	136	93
Mg^{++} (mg/L)	38.2	38.8	38.2	38.2	35.4	35.6	17.3	29.1	32.7	32.0	17.7
Alcalinity	425	430	425	430	425	425	425	320	430	420	340
CO_2 (mg/L)	23.3	23.8	26.4	23.8	18.5	22.0	10.6	14.1	12.3	13.6	5.3
Fe (total) (mg/L)	0.4	0.3	0.4	0.4	0.1	0.2	0.5	0	0	0	0.5
Fe^{++} (mg/L)	0	0	0	0	0	0	0.4	0	0	0	0.5
Fe^{+++} (mg/L)	0.4	0.3	0.4	0.4	0.1	0.2	0.1	0	0	0	0

* = mg/L $CaCO_3$

TABLE IV – Chemical-physical properties of water samples taken at the various stations on the 08/05/91; L=left bank – R=right bank

8/5/91	Station 1		Station 2		Station 3		Station 4		Station 5		CAV.
	S	D	S	D	S	D	S	D	S	D	
T °C (air)	11	11	15	15	17	17	20	18	20	18	20
T °C (water)	14	14	14	14	14	14	16	14	14	14	16
pH	7.13	7.17	7.25	7.18	7.28	7.20	7.68	7.34	7.35	7.42	7.82
O_2 (mg/L)	0.3	0.4	0.6	0.6	0.6	0.6	0.4	1.0	1.0	1.0	0.4
NO_2^- (mg/L)	>2.0	>2.0	>2.0	>2.0	>2.0	>2.0	0	0.8	0.6	0.6	0
NH_4^+ (mg/L)	1.3	2.0	0.65	1.3	0.75	1.5	6.0	0.5	0.2	0.4	3.0
Cl^- (mg/L)	150	150	120	120	75	75	300	50	50	50	>300
Hardness*	502	477	472	488	456	473	425	441	438	445	409
Ca^{++} (mg/L)	136	127	134	134	132	130	127	124	124	124	127
Mg^{++} (mg/L)	38.9	38.3	32.8	36.6	30.2	35.6	25.9	31.5	30.7	32.4	22.0
Alcalinity	475	475	460	465	445	445	450	420	415	420	625
CO_2 (mg/L)	30.4	27.3	22.4	26.4	19.4	24.2	8.0	16.3	15.8	13.2	7.0
Fe (total) (mg/L)	0.5	0.4	0.4	0.3	0.2	0.1	0.3	0.1	trace	trace	0.4
Fe^{++} (mg/L)	0.3	0.3	0.1	trace	trace	trace	0.2	trace	0.1	0.1	0.2
Fe^{+++} (mg/L)	0.2	0.1	0.3	0.2	0.1	trace	0.1	trace	0	0	0.2

* = mg/L $CaCO_3$

TABLE V – Chemical-physical properties of water samples taken at the various stations on the 26/06/91; L=left bank – R=right bank

26/6/91	Station 1		Station 2		Station 3		Station 4		Station 5		CAV.
	S	D	S	D	S	D	S	D	S	D	
T °C (air)	21	21	24	24	27	27	29	29	29	29	29
T °C (water)	17	17	17	17	17	17	17	15	15	15	17
pH	7.37	7.21	7.38	7.38	7.54	7.42	7.54	7.54	7.50	7.53	7.52
O_2 (mg/L)	–	–	–	–	–	–	–	–	–	–	–
NO_2^- (mg/L)	>2.0	>2.0	>2.0	>2.0	>2.0	>2.0	0.1	0.5	0.5	0.5	0.1
NH_4^+ (mg/L)	2.0	2.0	2.0	2.0	1.3	1.5	>8.0	0.4	0.3	0.4	>8.0
Cl^- (mg/L)	150	150	150	150	150	150	>300	75	75	75	>300
Hardness*	502	463	474	473	456	463	445	429	437	436	434
Ca^{++} (mg/L)	133	128	124	124	127	127	124	123	123	123	126
Mg^{++} (mg/L)	40.7	34.3	39.2	39.2	33.2	34.9	32.4	29.2	30.9	30.9	28.6
Alcalinity	490	480	460	460	440	450	500	420	420	420	500
CO_2 (mg/L)	18.0	25.0	16.3	16.3	11.0	14.5	12.3	10.1	11.4	11.4	12.7
Fe (total) (mg/L)	0.3	0.4	0.2	0.2	0.1	0.2	0.6	0	0	0	0.6
Fe^{++} (mg/L)	0	0	0	0	0	0	0.5	0	0	0	0.5
Fe^{+++} (mg/L)	0.3	0.4	0.2	0.2	0.1	0.2	0.1	0	0	0	0.1

* = mg/L $CaCO_3$

The values of NH_3, of H_2S and NO_2^- increased from winter to summer due to the seasonal work of many preserved food industries operating in that area and due to the decrease of O_2 concentrations caused by the temperature increase.

Faunistic-Ecological Results

A total of 2,226 animals belonging to 6 phyla (Platyhelminthes, Rotifera, Nematoda, Mollusca, Annelida, Arthropoda) and which could be grouped in 13 taxa were collected (Tab. VI).

Station 5 (Tab. VII) is located upriver from the confluence, where all of the 6 phyla are observed, and has the highest number of species. Besides a 33.5% of Nematoda -with the species *Dorylaimus* sp. and *Rhabdolaimus* sp.- a 18.6% of Rotifera – *Rotaria rotatoria*, *Branchionus angularis* and *Philodina* sp.- and a 16.3% of Dipterans (larva) – with the species *Chaoborus plumicornis* and *Chironomus* sp.- there is also a 12% of Copepods (*Ciclops strenuus*), a 5% of Gastropods among which we find the species *Physa* sp., *Planorbis* sp. and *Physa fontinalis* – and a 2.4% of Hemipterans (*Velia rivorum*). The difference in the faunistic composition of the two

TABLE VI – Global structure of the fauna during the various samplings

TAXA	01/10/90	%	12/12/90	%	26/02/90	%	08/05/91	%	26/06/91	%	tot.	tot. %
Turbellaria	94	23.86	28	7.27	60	12.00	22	3.44	29	9.42	233	10.47
Rotifera	82	20.81	75	19.48	121	24.20	97	15.18	64	20.78	439	19.72
Nematoda	181	45.94	195	50.65	194	38.80	317	49.61	123	39.94	1010	45.37
Gastropoda	8	2.03	8	2.08	7	1.40	17	2.66	5	1.62	45	2.02
Oligochaeta	4	1.02	8	2.08	14	2.80	13	2.03	16	5.19	55	2.47
Hirudinea	3	0.76	2	0.52	5	1.00	1	0.16	10	3.25	21	0.94
Acarina	0	0.00	9	2.34	10	2.00	4	0.63	1	0.32	24	1.08
Cladocera	0	0.00	3	0.78	1	0.20	4	0.63	1	0.32	9	0.40
Ostracoda	2	0.51	1	0.26	3	0.60	3	0.47	15	4.87	24	1.08
Copepoda	13	3.30	34	8.83	49	9.80	37	5.79	8	2.60	141	6.33
Branchiopoda	1	0.25	2	0.52	1	0.20	2	0.31	0	0.00	6	0.27
Hemiptera	3	0.76	1	0.26	7	1.40	5	0.78	7	2.27	23	1.03
Diptera	3	0.76	19	4.94	28	5.60	117	18.31	29	9.42	196	8.81
TOTAL	394	100	385	100	500	100	639	100	308	100	2226	100
TOTAL (%)	17.70		17.30		22.46		28.71		13.84			100

TABLE VII – Composition of the fauna at station 5 at the various times of sampling

	01/10/90				12/12/90				26/02/91				08/05/91				26/06/91					
TAXA	St. 5S		St. 5D		St. 5S		St. 5D		St. 5S		St. 5D		St. 5S		St. 5D		St. 5S		St. 5D		tot.	tot. %
Turbellaria		0	1	1.1		0	2	2.25	1	1.18		0	2	2.9		0	3	10		0	9	1.09
Rotifera	10	16.9	24	26.4	5	10	23	25.8	10	11	45	45.5	7	10.1	21	9.95	4	13.3	4	10	153	18.59
Nematoda	36	61	49	53.8	12	24	27	30.3	19	22.4	13	13.1	18	26.1	95	45	3	10	4	10	276	33.54
Gastropoda	2	3.39	6	6.59	4	8	3	3.37	1	1.18	4	4.04	3	4.35	14	6.64	1	3.33	3	7.5	41	4.98
Oligochaeta		0	2	2.2	2	4	4	4.49		0	5	5.05	3	4.35	6	2.84		0	7	17.5	29	3.52
Hirudinea	2	3.39	1	1.1		0	2	2.25	3	3.53	1	1.01		0	1	0.47	3	10	5	12.5	18	2.19
Acarina		0		0	8	16		0	5	5.88	2	2.02	3	4.35		0	1	3.33		0	19	2.31
Cladocera		0		0	2	4	1	1.12	1	1.18		0		0	2	0.95		0		0	6	0.73
Ostracoda		0	2	2.2	1	2		0	3	3.53		0		0	1	0.47	2	6.67	4	10	13	1.58
Copepoda	5	8.47	3	3.3	7	14	14	15.7	25	29.4	19	19.2	7	10.1	11	5.21	6	20	2	5	99	12.03
Branchiopoda	1	1.69		0		0	2	2.25	1	1.18		0	2	2.9		0		0		0	6	0.73
Hemiptera	2	3.39	1	1.1	1	2		0	4	4.71	3	3.03		0	4	1.9		0	5	12.5	20	2.43
Diptera	1	1.69	2	2.2	8	16	11	12.4	12	14.1	7	7.07	24	34.8	56	26.5	7	23.3	6	15	134	16.28
TOTAL	59	100	91	100	50	100	89	100	85	100	99	100	69	100	211	100	30	100	40	100	283	100
TOTAL (%)		7.17		11.06		6.08		10.81		10.33		12.03		8.38		25.64		3.65		4.86		100

banks caused by the confluence of the Cavaiola stream can be noted at station 4 (Tab. VIII); as a matter of fact, we can say that among all the samples taken on the left bank, the Nematoda were the only animals found in mud samples. At station 3 (Tab. IX) and 2 (Tab. X) there are only Turbellarians – with the species *Microstomum lineare*, *Microstomum sp.*, *Stenostomum sp.*, *Mesostoma lingua*, *Mesostoma sp.*, Rotifera – with the species *Rotaria neptunia*, *Branchionus sp.*, Nematoda, with *Rhabditis limnicola*, Oligochaetes – with the species *Tubifex tubifex*, *Limnodrilus sp.*, *Stylaria sp.* and Dipterans – once again with larva of *Chironomus sp.*, *Chironomus thumnii*, *Eristalis sp.*-. At station 1, besides the same taxa found at stations 2 and 3 there were also occasional findings of some Copepods (Tab. XI).

Discussion

The contemporary presence of ammonium ion and nitrous nitrogen shows that the pollution of the Sarno river is partially of organic origin (sewers, preserved food industry drains, urban waste, farm rejects, etc.) even though toxic inorganic substances due to minor tanning products are also present.

Despite the above, the pH value is slightly basic at all the stations. It is our opinion that many anthropogenic aspects concur to this, such as H_2S-buffered NH_3, both originated by the decomposition of organic matter or by preserved food industry drains, and anion HS^-, which in favourable condition is balanced with hydrogen sulphide and may thus dissociate itself a second time giving rise to S^{2-}, both originated by the decomposition of sulphide and the drains of some tannery processes using Na_2S.

Anomalous increases of the Cl^- ion concentration (probably due to the hide desalting process) were reported during the sampling both in the Cavaiola stream and on the left bank of station 4.

The concentration of ammonia also contributes to the definition of the pH; in water it reacts as follows:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

water will be more basic if ammonia increases; this reaction is completely moved to the left.

An increase of the ammonia ion in time has been observed. Its utmost level was reached in the sampling carried out on the 26th of June, 1991;

TABLE VIII – Composition of the fauna at station 4 at the various times of sampling

	01/10/90				12/12/90				26/02/91				08/05/91				26/06/91					
TAXA	St. 4S		St. 4D		St. 4S		St. 4D		St. 4S		St. 4D		St. 4S		St. 4D		St. 4S		St. 4D		tot.	tot. %
Turbellaria		0	51	59.3		0		0		0	2	3.64		0	4	3.39		0	2	4.65	59	12.94
Rotifera		0	29	33.7		0	2	5.13		0	21	38.2		0	37	31.4		0	3	6.98	92	20.18
Nematoda	39	100	1	1.16	15	100	20	51.3	11	100	17	30.9	43	100	31	26.3	7	100	11	25.6	195	42.76
Gastropoda		0		0		0	1	2.56		0	2	3.64		0		0		0	1	2.33	4	0.88
Oligochaeta		0		0		0	2	5.13		0	3	5.45		0		0		0	6	14	11	2.41
Hirudinea		0		0		0		0		0	1	1.82		0		0		0	2	4.65	3	0.66
Acarina		0		0		0	1	2.56		0	3	5.45		0	1	0.85		0		0	5	1.10
Cladocera		0		0		0		0		0		0		0	2	1.69		0	1	2.33	3	0.66
Ostracoda		0		0		0		0		0		0		0	2	1.69		0	9	20.9	11	2.41
Copepoda		0		0		0		0		0		0		0	2	1.69		0	9	20.9	11	2.41
Branchiopoda		0	5	5.81		0	13	33.3		0	4	7.27		0	19	16.1		0		0	41	8.99
Hemiptera		0		0		0		0		0		0		0		0		0		0	0	0.00
Diptera		0		0		0		0		0		0		0	1	0.85		0	2	4.65	3	0.66
TOTAL	39	100	86	100	15	100	39	100	11	100	55	100	43	100	118	100	7	100	43	100	456	100
TOTAL (%)		8.55		28.86		4.39		8.55		2.41		12.06		9.43		25.88		1.54		9.43		100

TABLE IX- Composition of the fauna at station 3 at the various times of sampling

	01/10/90				12/12/90				26/02/91				08/05/91				26/06/91					
TAXA	St. 3S		St. 3D		St. 3S		St. 3D		St. 3S		St. 3D		St. 3S		St. 3D		St. 3S		St. 3D		tot.	tot. %
Turbellaria	6	25	1	8.33	7	25		0	12	34.3	9	19.1	6	16.2		0	1	5.88	3	5.66	45	14.15
Rotifera	3	12.5	6	50	2	7.14	7	23.3	11	31.4	14	29.8	9	24.3	1	2.86		0	12	22.6	65	20.44
Nematoda	13	54.2	5	41.7	19	67.9	23	76.7	10	28.6	21	44.7	21	56.8	25	71.4	13	76.5	36	67.9	186	58.49
Gastropoda		0		0		0		0		0		0		0		0		0		0	0	0.00
Oligochaeta	2	8.33		0		0		0		0		0		0	3	8.57		0		0	5	1.57
Hirudinea		0		0		0		0		0		0		0		0		0		0	0	0.00
Acarina		0		0		0		0		0		0		0		0		0		0	0	0.00
Cladocera		0		0		0		0		0		0		0		0		0		0	0	0.00
Ostracoda		0		0		0		0		0		0		0		0		0		0	0	0
Copepoda		0		0		0		0		0		0		0		0		0		0	0	0.00
Branchiopoda		0		0		0		0		0		0		0		0		0		0	0	0.00
Hemiptera		0		0		0		0		0		0		0		0		0		0	0	0.00
Diptera		0		0		0		0	2	5.71	3	6.38	1	2.7	6	17.1	3	17.6	2	3.77	17	5.35
TOTAL	24	100	12	100	28	100	30	100	35	100	47	100	37	100	35	100	17	100	53	100	318	100
TOTAL (%)		7.55		3.77		8.81		9.43		11.01		14.78		11.64		11.01		5.35		16.67		100

TABLE X – Composition of the fauna at station 2 at the various times of sampling

	01/10/90				12/12/90				26/02/91				08/05/91				26/06/91					
TAXA	St. 2S		St. 2D		St. 2S		St. 2D		St. 2S		St. 2D		St. 2S		St. 2D		St. 2S		St. 2D		tot.	tot. %
Turbellaria	13	52		0		0	9	22.5	12	18.2	8	21.1	6	25	3	7.89	1	5.88	3	5.66	55	15.71
Rotifera		0	3	30	8	20.5	15	37.5	9	13.6	4	10.5	2	8.33	5	13.2		0	12	22.6	58	16.57
Nematoda	12	48	7	70	31	79.5	16	40	43	65.2	23	60.5	16	66.7	28	73.7	13	76.5	36	67.9	255	64.29
Gastropoda		0		0		0		0		0		0		0		0		0		0	0	0.00
Oligochaeta		0		0		0		0		0	3	7.89		0	1	2.63		0		0	4	1.14
Hirudinea		0		0		0		0		0		0		0		0		0		0	0	0.00
Acarina		0		0		0		0		0		0		0		0		0		0	0	0.00
Cladocera		0		0		0		0		0		0		0		0		0		0	0	0.00
Ostracoda		0		0		0		0		0		0		0		0		0		0	0	0
Copepoda		0		0		0		0		0		0		0		0		0		0	0	0.00
Branchiopoda		0		0		0		0		0		0		0		0		0		0	0	0.00
Hemiptera		0		0		0		0		0		0		0		0		0		0	0	0.00
Diptera		0		0		0		0	2	3.03		0		0	1	2.63	3	17.6	2	3.77	8	2.29
TOTAL	25	100	10	100	39	100	40	100	66	100	38	100	24	100	38	100	17	100	53	100	350	100
TOTAL (%)		7.14		2.86		11.14		11.43		18.86		10.86		6.86		10.86		4.86		15.14		100

TABLE XI – Composition of the fauna at station 1 at the various times of sampling

	01/10/90		12/12/90		26/02/91		08/05/91		26/06/91			
TAXA	St. 1S	St. 1D	St. 1S	St. 1D	St. 1S	St. 1D	St. 1S	St. 1D	St. 1S	St. 1D	tot.	tot. %
Turbellaria	13 41.9	9 52.9	2 22.2	2 10.5	3 14.3	13 30.2	0	1 4.55	2 6.45	10 50	61	21.63
Rotifera	7 22.6	0	13 36.1	0	2 9.52	5 11.6	8 19	7 31.8	11 35.5	0	53	18.79
Nematoda	11 35.5	8 47.1	15 41.7	17 89.5	14 66.7	23 53.5	31 73.8	9 40.9	14 45.2	7 35	149	52.84
Gastropoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Oligochaeta	0	0	0	0	1 4.76	2 4.65	0	0	0	2 10	5	1.77
Hirudinea	0	0	0	0	0	0	0	0	0	0	0	0.00
Acarina	0	0	0	0	0	0	0	0	0	0	0	0.00
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0.00
Ostracoda	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0.00
Branchiopoda	0	0	0	0	1 4.76	0	0	0	0	0	1	0.35
Hemiptera	0	0	0	0	0	0	0	0	0	0	0	0.00
Diptera	0	0	0	0	0	0	3 7.14	5 22.7	4 12.9	1 5	13	4.61
TOTAL	31 100	17 100	36 100	19 100	21 100	43 100	42 100	22 100	31 100	20 100	282	100
TOTAL (%)	10.99	6.03	12.77	6.74	7.45	15.25	14.89	7.80	10.99	7.09		100

this may be ascribed to an increase in the decomposition of organic sublayes, also responsible for the high concentration of sulphide hydrogen.

The presence of hydrogen was observed both through a qualitative analysis and an organoleptic analysis (in accordance with the method proposed by Bianucci *et al.*, 1980). The last sample revealed an increase of H_2S too.

Besides the decomposition, sulphides are also originated by the drains of some tan manufacturing processes; in particular by hair-removal and by the softening of hides. As a matter of fact, the substance used for this process is Na_2S, a sulphide which is easily soluble in water:

(1) $$Na_2S_{(s)} + H_2O = 2Na^+ + HS^- + OH^-$$

From this reaction we can observe that also anion HS^- is obtained, which is in balance with hydrogen sulphide:

(2) $$HS^- + H_2O \rightleftharpoons H_2S + OH^-$$

or in favourable conditions, anion HS^- can give rise to a second dissociation causing the reaction:

(3) $$HS^- + H_2O \rightleftharpoons H_3O^+ + S^{2-}$$

In the case of the Sarno river the (2) reaction takes place, thus contributing to a stabilization of the pH on slightly basic values.

The conditions of the river worsen in summertime due to its decreased flow rate and to the higher temperatures causing oxygen to be less soluble in water (oxygen is already close to 0 mg/L even in normal conditions. The decrease of oxygen, in turn, causes the putrefaction and anaerobic digestion.

The high value of total hardness is not due, as expected, to the inflow of the Cavaiola stream, as values are already high at station 5. The results obtained show indeed that the waters of the Sarno river are "very hard" because they are originated in calcareous substrata.

The number of animals is not particularly low, even though it is distributed among the different taxa. Furthermore, we observed that the main part of the widely diversified benthofauna (13 taxa) can only be found at station 5 located upriver from the Cavaiola stream, which is the main source of pollution.

The species found towards the mouth of the river belong all to polysaprobic habitats with a very low request of O_2 (0.4 mg/L).

The analysis of the tested indexes confirms the above said too. As a matter of fact, the Dominance index of Simpson shows how the whole period of sampling was characterized by a sharp separation between the parts upstream and downstream from the confluence with the Cavaiola stream; Dominance values near station 4S are, in fact, close to 1 (Fig. 2 & 3).

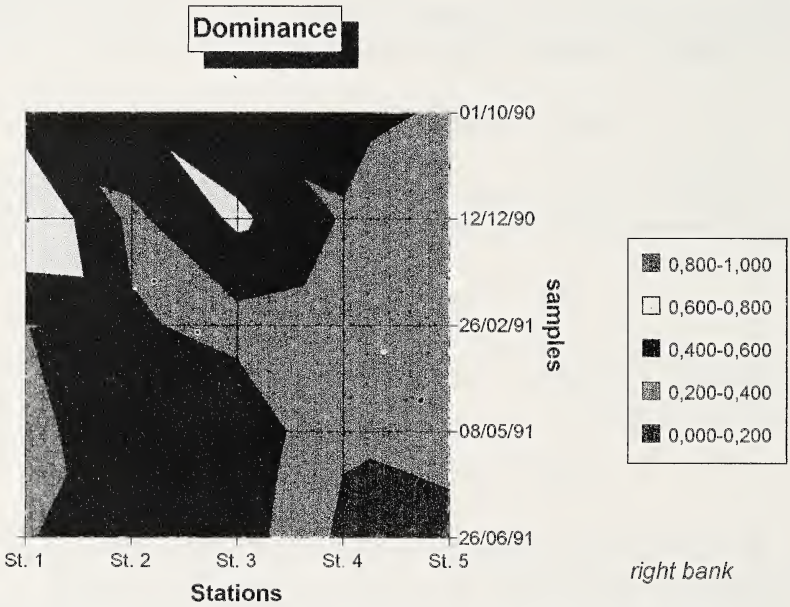

FIGURE 2 – Graphic representation of the Dominance index

The Difference calculated in accordance with the index of Shannon gave very low values at station ST 4L while the highest values of difference (Fig. 4 & 5) were obtained at station 5. At the beginning of the summer we didn't observe the expected increase in the difference, and this is likely due to the increase of drains mainly caused by the seasonal activity of preserved food industries.

Also the Equality index calculated on the basis of the Diversity index of Shannon confirms the missed improvement of populations in summer time, as shown in the diagrams (Fig. 6 & 7).

The best sampling period is in May, i.e. when preserved food industries are not yet fully operating, while even the sampling values of station 5 worsen in June.

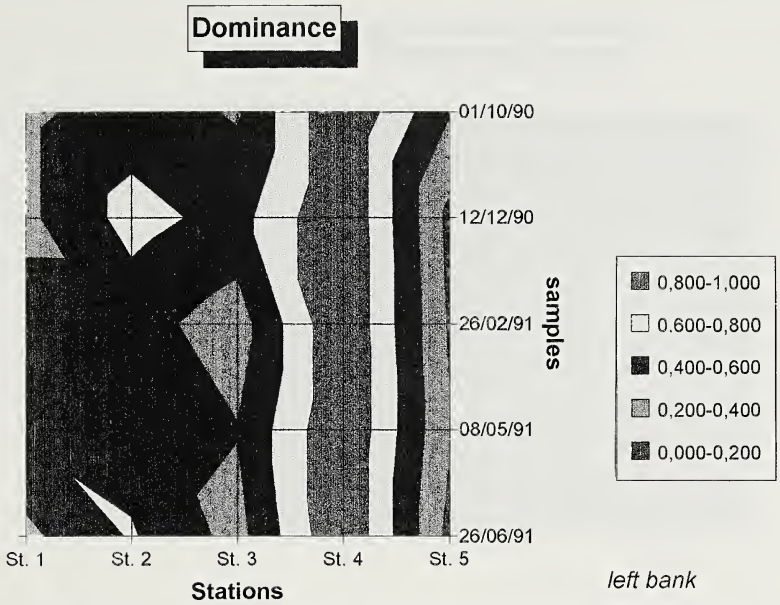

FIGURE 3 – Graphic representation of the Dominance index

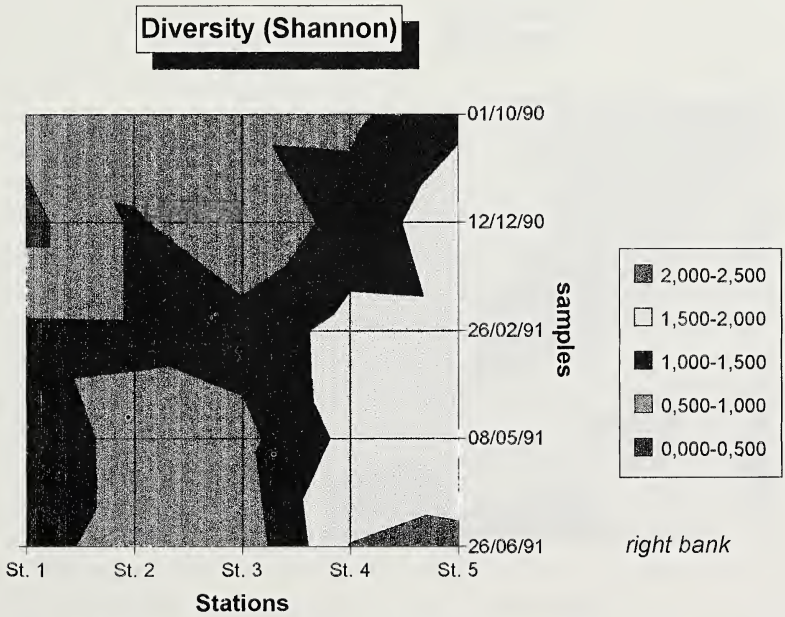

FIGURE 4 – Graphic representation of the Diversity index of Shannon

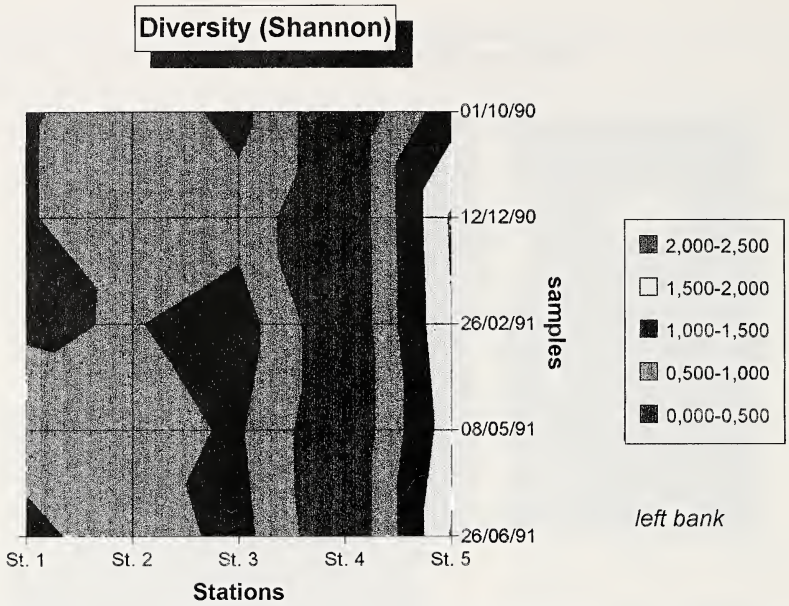

FIGURE 5 – Graphic representation of the Diversity index of Shannon

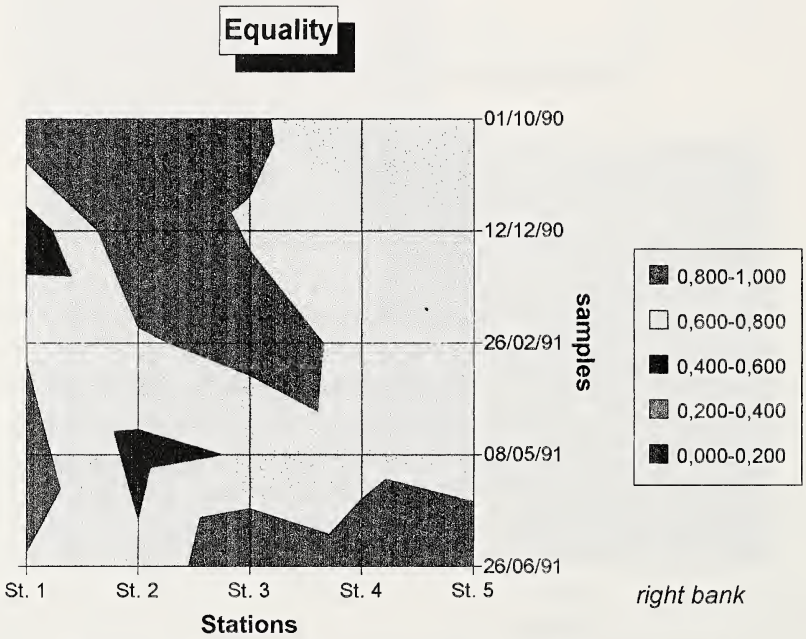

FIGURE 6 – Graphic representation of the Equality index

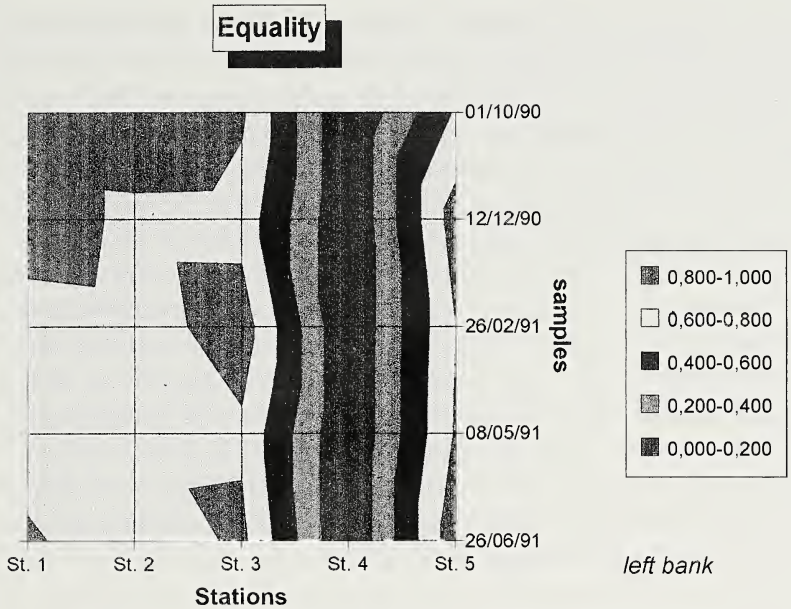

FIGURE 7 – Graphic representation of the Equality index

River pollution due to preserved food industries must therefore be added to the more serious and steady contribution of tanning industries.

The different Dominance, Equality and Diversity index values suggest that the part of river between the inflow of the Cavaiola stream and the mouth of the river is the most damaged, even if a light recovery is observed in its terminal part. In fact, according to Odum (1973), the Diversity index is low in physically controlled ecosystems, i.e. subject to highly restrictive chemical-physical factors, while it is high in biologically controlled ecosystems. Thus, a higher Diversity means more complex feeding networks, more cases of symbiosis and more possibilities of a negative feedback control, which reduces variations and increases stability.

The Diversity index of Shannon clearly shows this zonation of populations too.

In view of the above, it can thus be stated that along the examined part of the Sarno river it is possible to identify three areas with different species and different quantities of animals collected for each species.

The first area can be identified by stations 5 and 4L. It is the richest in phyla and its faunistic structure can be included among the mesosaprobic alpha and beta classes of V. Sladecek's system (1965). The second area is

the most damaged by the pollution. It includes the part downstream from the inflow of the Cavaiola river and can be defined as metasaprobic. The third and last area can be identified by stations 3, 2 & 1. This area has strong polisaprobic features.

Having used research methods adopted in some previous studies (Battaglini *et al.*, 1968, 1971, 1979), the conditions of the Sarno river in 1979 could be compared with its current conditions.

Compared data show a general worsening in the conditions of the river. Polluting elements cannot be eliminated, thus causing the accumulation of toxic mud on the bottom and the subsequent moving away from normal conditions, i.e. conditions preventing the growth up of a typical river environment benthofauna. Significant seasonal variations of waste are another obstacle to the formation of well defined biocoenose (d'Elia, 1988). The pH is almost unchanged, but NH_4^+ and nitrous nitrogen concentrations have increased, confirming a higher inflow rate of organic origin. Even more worrying is the fall of the dissolved oxygen concentration from mean values of approximately 3.3 mg/L in 1979 to approximately 0.6 mg/L. This is likely to be the cause of the decreased specific Diversity. As regards total hardness, the concentration doubled at all stations and in all samplings.

The worsening and the increase in the concentration of polluting substances is also caused by the increased exploitation of the springs, so that most of the spring waters are conveyed to be used as drinking water.

Considering animal communities as a whole, the first things to be noticed are the strong decrease in the number of taxa and the lower number of single animals. The disappearance of some taxa, such as Gastropods, is clearly due to the worsening of all environmental conditions. Only Dipterans larva were collected among the Arthropoda. Some particularly resistant species as Turbellari (*Stenostomum* sp., *Mesostoma* sp., *Microstomum* sp.), Rotifera (*Rotaria neptunia*) and Nematoda can still be found in the river. The species variety is heavily reduced among the taxa too. Only species typical of metasaprobic or, at the most, polysaprobic environments can survive (Liebmann, 1951). As a matter of fact, the only species of Oligochaeta found is the *Tubifex tubifex*.

The zoocoenose of the downstream part and up to the mouth of the Sarno river consists of few species – all adapted to typical polysaprobic environment conditions – whose distribution is determined by the inflow of the Cavaiola stream. The faunistic data are perfectly consistent with the

chemical-physical parameters and lead to the same two basic types of polluting reasons: the massive number of industries and urban waste.

Station 5 still maintains acceptable conditions confirmed by a not very high Dominance value and an almost sufficient Diversity. From the confluence of the Cavaiola stream onwards there is a "zonation" even in the case of the little Sarno river. In fact, the waters of this tributary are a real ecological barrier against the diffusion of animals. Dominance indexes rise substantially while Diversity indexes decrease, thus revealing the highly stressing condition.

The comparison between the current data and those previously collected by one of the Authors (Battaglini *et al.*, 1968) shows a general worsening of the conditions of the river. Anthropogenic polluting elements cannot be eliminated, thus causing the accumulation of toxic mud on the bottom and the subsequent moving away from normal conditions. All this prevents the growth of a typical river environment benthofauna, also because of the strong seasonal variations in the type and quantity of waste.

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Bionomics of *Myopites stylata* F. (Diptera, Tephritidae) and its natural enemies in Vivara island (Gulf of Naples)

Fimiani P., Digilio M.C.[1]

Dipartimento di Biologia, Difesa e Biotecnologie agro-forestali,
Università degli Studi della Basilicata, Via Nazario Sauro 85, 85100 Potenza, Italy
Dipartimento di Entomologia e Zoologia agraria, Università degli Studi di Napoli «Federico II»
Via Università 100, 80055 Portici (Na), Italy

Key words: *Inula*, host alternation, *Bactrocera (Dacus) oleae*.

Abstract. *Myopites stylata* F. (Diptera, Tephritidae), is a gall-forming fly living on the composite *Inula viscosa* Aiton. The bionomy and parasitoid complex of this insect species were studied in Vivara, a small island in the Gulf of Naples. *M. stylata* adults always emerged from the second half of September to the end of October. The parasitoid species obtained were different Chalcidoids (Hymenoptera): two undetermined species of *Habrocytus* Thomson, belonging to *albiformis* Walker group (Pteromalidae), *Eupelmus urozonus* Dalman (Eupelmidae), *Torymus cyanimus* Boheman and *Dimeromicrus kiesenwetteri* Mayr (Torymidae), *Eurytoma tibialis* Boheman (Eurytomidae). The role and effectiveness of *E. urozonus* as a possible natural control agent of the Olive Fly, *Bactrocera* (*Dacus*) *oleae* (Gmelin) is at present not well defined. However, in this study *M. stylata*, compared with other Mediterranean areas, resulted an important alternative host for *E. urozonus*.

Riassunto. Bionomia di *Myopites stylata* F. (Diptera, Tephritidae) e dei suoi nemici naturali nell'isola di Vivara (Golfo di Napoli).

Il presente lavoro riporta alcuni dati bionomici su *Myopites stylata* F. (Diptera, Tephritidae) e sul complesso dei suoi nemici naturali nell'isola di Vivara (Golfo di Napoli). Lo studio è stato effettuato mediante raccolta dei capolini fiorali della Composita *Inula viscosa* Aiton, trasformati in galle dall'azione del Dittero. Dalle galle sono sfarfallati il Tefritide ed i parassitoidi relativi, tutti appartenenti alla superfamiglia Chalcidoidea (Hymenoptera). Le specie ottenute sono: *Torymus cyanimus* Boheman e *Dimeromicrus kiesenwetteri* Mayr (Torymidae), *Eurytoma tibialis* Boheman (Eurytomidae), due specie non determinate del genere *Habrocytus* Thomson, appartenenti al gruppo *albiformis* Walker (Pteromalidae), e *Eupelmus urozonus* Dalman (Eupelmidae). I parassitoidi sono sfarfallati lungo un arco di tempo piuttosto lungo, mentre gli adulti del Dittero nel periodo settembre-ottobre, coincidente con la fioritura dell'inula a Vivara. Il parassitoide quantitativamente più importante è risultato *Habrocytus* spp. Anche *E. urozonus* si ritrova in quantità notevole, contrariamente ad altre aree mediterranee dove alcuni Autori hanno ottenuto pochi o nessun esemplare di questo parassitoide dalle galle di *I. viscosa*. A Vivara, quindi, *M. stylata* costituisce un importante ospite alternativo di *E. urozonus*, che potrebbe quindi risultare utile, nonostante le complesse intera-

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zioni con gli altri parassitoidi, nel controllo naturale della Mosca dell'olivo, *Bactrocera (Dacus) oleae* (Gmelin).

Introduction

The mediterranean maquis in Vivara island (Gulf of Naples) is a natural environment free of antropic influence, which offers interesting opportunities for ecological studies (Fimiani, 1977; 1981). The features of this island are reported in a special issue edited by Regione Campania (1981).

A list of the insect species living on Vivara island, collected with sweeping nets, is available (D'Antonio & Fimiani, 1988).

Myopites stylata F. (Diptera, Tephritidae) lives exclusively on the composite *Inula viscosa* Aiton, where it produces galls from flowers (Fig. 1). *I. viscosa* is common in the Mediterranean area, where it is often associated with olive groves. *Myopites* females typically lay their eggs in flower ovaries, by introducing the ovipositor through the corollar tube. The hatching larva penetrates into the flower receptacle, which gradually becomes hypertrophic, and finally is transformed in a wooden-plurilocular gall, with special tubercles in place of the achenes.

The association between inules and olive trees was investigated in Italy by Martelli (1910), Bua (1938) and Silvestri (1940), then in Greece by Isaakidès (1955; 1957), in Corse (Féron *et al.*, 1961) and continental France (Delanoue & Arambourg, 1965), and more recently in Crete (Neuenschwander *et al.*, 1983). The importance of ecological studies on the relationship between spontaneous vegetation and olive tree is also recently emerged in the last OILB Working Group on Fruit Flies, held in Sassari in November 1990 (unpubl.).

The present note aims at defining the natural enemy complex of *M. stylata* in Vivara, trying also to assess in this environment the role of inule as a natural reservoir of *Bactrocera* (*Dacus*) *oleae* (Gmelin) parasitoids in olive orchards.

Materials and methods

The galls were collected once a year from 1982 to 1985, in October, January, November and May respectively in the four years of the study. The samples were kept in the laboratory for over one year to recover all the

emerging adult flies and parasitoids. In 1982, galls were cumulatively stored in plastic boxes (cm 20 x 30 x 10) closed with a fine gauze. For all the

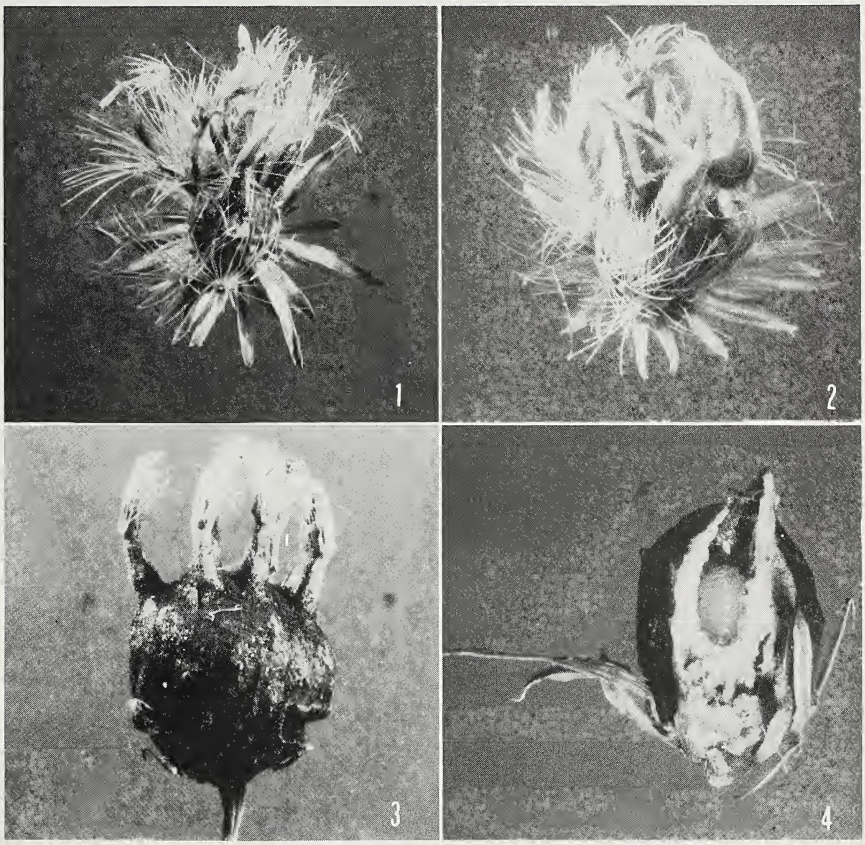

FIGURE 1 – *Myopites stylata* F. gall on *Inula viscosa* Aiton. 1, 2. Gall formation. 3. Gall completely formed. 4. *M. stylata* mature larva.

remaining years of the study the galls were individually stored in glass vials (cm 10 x 0.6) plugged with cotton wool. Part of the collected galls were also dissected. Adults emerging from galls stored in the boxes were periodically collected for species identification, while those isolated in the vials were weekly collected to assess the time distribution of adult emergence trends.

RESULTS AND DISCUSSION

The total number of both fly and parasitoid specimens obtained in the different years of study is reported in Table I. The parasitoid complex is

illustrated in Table II. The relative abundance of parasitoids emerging from the galls is shown in Table III. For *M. stylata* a sex ratio nearly equal to 0.5 was recorded (Tab. I). The adults of this species mostly emerged

TABLE I – *Myopites stylata* and parasitoids emerged from collected galls.

collecting years	1982		1983		1984		1985	
collected galls	530		159		80		43	
active galls	*		124		59		43	
Number per sex	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Myopites stylata F.	176	151	99	92	40	30	26	20
total parasitoids	668	484	64	130	56	31	13	21
Habrocytus spp.	618	421	40	82	45	27	4	7
Eupelmus urozonus Dalman	13	28	6	21	–	–	9	13
Torymus ?cyanimus Boheman	8	10	11	11	4	1	–	1
Dimeromicrus kiesenwetteri Mayr	8	14	5	13	6	2	–	–
Eurytoma tibialis Boheman	21	11	2	3	1	1	–	–

* For this collection it was not possible to know the number of active galls, i.e. the galls yielding adults, both flies or parasitoids.

during the second and third week of September (Figs. 2, 3), apart from a few individuals emerging through October. For isolated galls it was possible to assess that in average several flies and parasitoids were in most cases present for each inhabited gall (Tab. IV). However, the galls which did not yield any insect, not even the Tephritid who had caused the gall formation, were 22% in 1983 and 26% in 1984 (Tab. I). In fact, upon dissection of galls collected in November, we observed that in some cases they were absolutely empty.

Pteromalids

Two species of *Habrocytus* Thomson were obtained, differing in marginal vein length and slightly in colour.

Habrocytus spp. was usually the most abundant species (Tab. III). Its relative abundance ranged from 32.3% up to 90.2%, and was always the highest except in May 1985, when *Eupelmus urozonus* Dalman exceeded

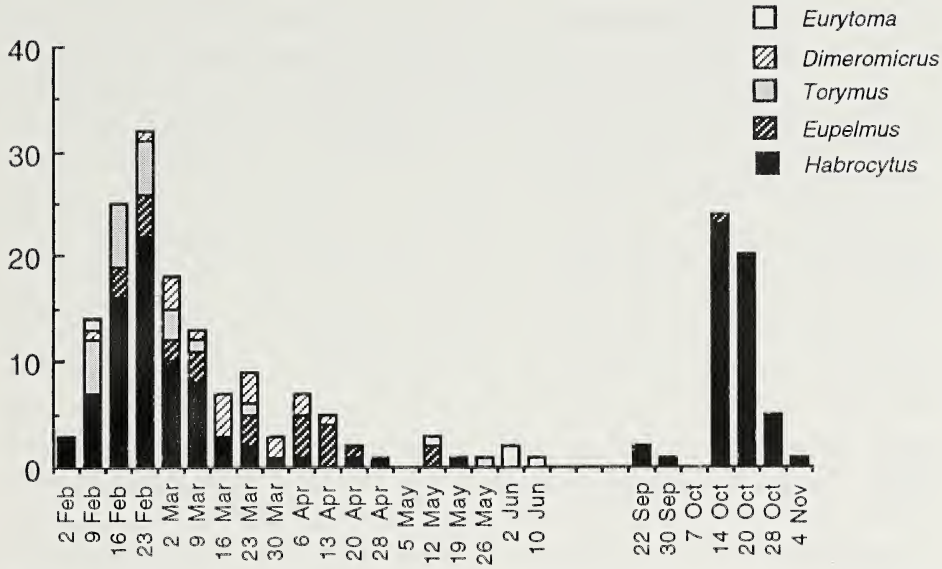

FIGURE 2 – Emergences of *Myopites stylata* parasitoids. Collection of January 1983.

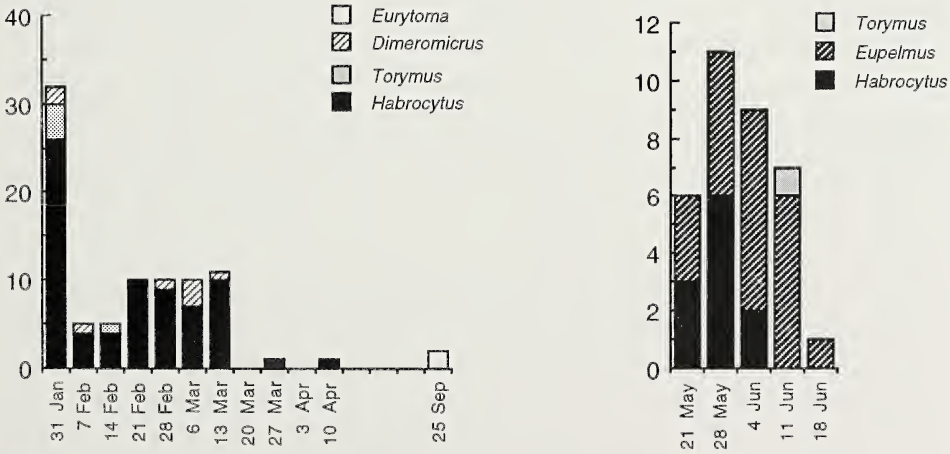

FIGURE 3 – Emergences of *Myopites stylata* parasitoids.
Collection of November 1984 (left) and May 1985 (right). The galls of both these collections are produced by Tephritids emerged in September-October 1984.

TABLE II – The parasitoid complex.

parasitoid	family and subfamily	feeding behaviour	host
Habrocytus spp. *albiformis* Walker group	Pteromalidae Pteromalinae	ectophagous	polyphagous
Eupelmus urozonus Dalman	Eupelmidae Eupelminae	ectophagous	poliphagous
Torymus ?cyanimus Boheman	Torymidae Toryminae	ectophagous	Tefritid galls on Composites
Dimeromicrus kiesenwetteri Mayr	Torymidae Monodontomerinae	ectophagous	Tefritid galls on Composites
Eurytoma tibialis Boheman	Eurytomidae Eurytominae	endophagous	Tefritids galls on Composites

TABLE III – Relative abundance of parasitoids emerged from *Myopites stylata* galls

collecting years	1982	1983	1984	1985
Habrocytus spp.	90.19	62.89	82.76	32.35
Eupelmus urozonus Dalman	3.56	13.91	–	64.71
Torymus ?cyanimus Boheman	1.56	11.34	5.74	2.92
Dimeromicrus kiesenwetteri Mayr	1.91	9.28	9.2	–
Eurytoma tibialis Boheman	2.78	2.58	2.3	–

TABLE IV – Average number of *Myopites stylata* and parasitoids emerged from one gall.

collecting years	*M. stylata* $\bar{x} \pm sd$	parasitoids $\bar{x} \pm sd$	N.
1983	1.54 ± 1.62	1.57 ± 1.17	124
1984	1.19 ± 1.40	1.47 ± 1.18	59
1985	1.07 ± 1.08	0.79 ± 0.94	43

the number of Pteromalids present in our samples (Tab. III). The adult emergence was mainly registered from January until May (Fig. II, III) and, in 1983, to a lower extent also in September-October (Fig. 2).

Habrocytus trypetae Thomson is reported both as an ectoparasitoid of Tephritid larvae and as a hyperparasitoid on *Eurytoma curta* Walker (Varley, 1937). The possible occurrence of similar ecological relationships in the case of the *Habrocytus* spp. that we found in Vivara remains to be evaluated. In Central (Terracina, Latina, Lazio) and Southern Italy (Calabria) *Habrocytus* was already reported as the most common parasitoid found on *M. stylata* (Martelli, 1910). The high relative abundance of Pteromalids has been reported also by Féron *et al.* (1961) for Corsica. In this case an undetermined species of *Pteromalus* accounted for 40% of the total insects collected. In contrast, *Habrocytus* sp. was only rarely reared from *Myopites* galls collected in the Marche region (Rivosecchi, 1960a). However, this different situation could be partly due to the fact that the host species were *Myopites blotii* Brébisson and *M. frauenfeldi* Schiner.

Eupelmids

In this group, the only represented species is *Eupelmus urozonus* Dalman. The relative abundance of this species varies depending on the collection periods, reaching up to 64.7% (May 1985 collection, Tab. III). *E. urozonus* emerged at the same time as *Habrocytus*, with the last flights recorded in October (Figs. II, III). No *E. urozonus* is obtained from the November 1984 collection (Tab. III).

While some authors (Isaakidès, 1955, 1957) consider *M. stylata* as a valid alternative host for *E. urozonus*, others did not (Fèron *et al.*, 1961; Delanoue & Arambourg, 1965; Neuenschwander *et al.*, 1983). In fact, in Crete Neuenschwander *et al.* (1983) reported a low presence of this Chalcidoid (Tab. V). Their data are different from our findings (Tab. V). Fèron *et al.* (1961) reported that *M. stylata* galls collected in Corse in September did not yield any *E. urozonus*; but, because the absence of spontaneous vegetation resulted in a higher rate of olive fly infestation, they suggested a possible positive role of wild plants, such as inules, in olive groves, as natural reservoirs of *Eurytoma* sp. In Vivara too we found that no or few *E. urozonus* emerged from the samples collected in autumn (October 1982 and November 1984), then reports of Corse are not really in disagreement with Vivara ones.

TABLE V – *Eupelmus urozonus* adults emerged from 100 galls. ° from Neuenschwander *et al.*, 1983.

localities	ratios	dates of collection
Crete	2.98	December °
Vivara	7.74	October 1982
Vivara	16.98	January 1983
Vivara	51.16	May 1985

Delanoue & Arambourg (1965) found that *E. urozonus* presents a different oviposition behaviour on *M. stylata* and on *D. oleae*. The existence of biotypes adapted to different hosts would prevent a shift between the two Tephritids.

Torymids

Torymids were always present in low numbers in our samples, with a relative abundance ranging between 1.6% and 11.3% (Tab. 3). Adult emergences of Torymids species started in January, about at the same time as *Habrocytus*, and the highest values were registered in February-March (Figs. 2, 3). In Marche region (Rivosecchi, 1960b) *D*imeromicrus *kiesenwetteri* Mayr early in the season shows a few generations on *Myopites blotii* Brébisson and *M. frauenfeldi* Schiner, and afterwards it develops and overwinters on *M. stylata* galls on *I. viscosa*, since these galls are persistent during winter.

Varley (1941) observed in *Torymus cyanimus* Boheman a tendency to superparasitism: the female commonly lays many eggs in one host, leading to a high mortality rate, which might account for the low presence of *T. cyanimus* adults recorded in Vivara.

Superparasitism occurrence has been reported also for *D. kiesenwetteri*: adult females of this species were found to lay up to 10 eggs per host; *D. kiesenwetteri* successfully developed also as an ectophagous hyperparasite on *Eurytoma curta* larvae (Rivosecchi, 1960a). On the Adriatic coast (Rivosecchi, 1960b) *D. kiesenwetteri* is a common parasitoid of Tephritids, particularly of species of the genus *Myopites* Brébisson.

Eurytomids

Eurytoma tibialis Boheman (= *E. curta* Auct., nec Walker) was obtained only in a few specimens (Tab. III), which scatteredly emerged in different periods of the year (Figs. 2, 3).

The similarity of emergence times observed for parasitoids in the different years of the study, in spite of laboratory storage of galls, and, in most cases, the significant association with the life cycle of the host seem to suggest the possible existence of diapause. However, Neuenschwander *et al.* (1983) after recording the onset of *E. urozonus* emergence in December, concluded that diapause in Crete is less severe than in continental Greece (Louskas, in Neuenschwander *et al.*, 1983). Rivosecchi (1958) compared hibernating larvae of two parasitoids of *Tephritis stictica* Loew, *Eurytoma tristis* Mayr and *Ormyrus hungaricus* Erdös, kept at room temperature, at 25°C and at 30°C, and noticed that exposure to higher temperatures fastens the development rate, causing pupation to occur much in advance in respect to parasitoids stored at lower temperatures. For *M. stylata* the diapause regulated developmental arrest is much more evident. In fact *Myopites* pupae collected in Vivara in November, in galls not yet completely formed, did not develop into adults until the occurrence of *I. viscosa* flowering, only 10 months later.

For isolated galls it was possible to assess that in average several flies and parasitoids were in most cases present for each inhabited gall (Tab. 4).

In conclusion, the results show that in Vivara island *I. viscosa* can be an effective natural reservoir for *E. urozonus*, which might be useful in the natural control of the olive fly, *B. oleae*.

ACKNOWLEDGMENTS

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Immunocytochemistry of carbonic anhydrase in the chick embryo membranous labyrinth during development

Avallone B., Balsamo G., Marmo F.

Cattedra di Istologia ed Embriologia
Dipartimento di Genetica, Biologia generale e Molecolare
Università degli Studi «Federico II», Via Mezzocannone 8, 80134 Napoli, Italy

Key words: Immunocytochemistry, Carbonic-anhidrase, Embryo, Labyrinth.

Abstract. An immunocytochemical study with polyclonal antibodies was carried out with the aim to localize carbonic anhydrase (CA) isozymes CA I and CA II in the various cells of chick membranous labyrinth during development.

Our data indicate that CA I and CA II are localized in the same cells in the chick embryos. In fact CA I and CA II are diffusely localized in the membranous labirinth bud during the early developmental stages, successively the sensorial epithelium of the *maculae acusticae* and *crista ampullaris* strongly stained by the immunocytochemical procedure. Cochlear and vestibular dark cells consistently appeared stained for CA I and CA II. The epithelial cells of the endolymphatic sac and of the brain *choroid plexus* were similarly stained for both isozymes. In one day and one week chicken CA was visualized immunocytochemically in the same sites as at advanced embryonal stages. It is suggested that these findings are related to otolith formation and maintenance, as well as to fluid and ionic flux in the endolymph.

Riassunto. Studi Immunocitochimici sull'anidrasi carbonica del labirinto membranoso dell'embrione di pollo durante lo sviluppo.

È stato condotto uno studio con anticorpi policlonali allo scopo di localizzare gli isoenzimi dell'anidrasi carbonica (CA) CA I e CA II nel labirinto membranoso del pollo durante lo sviluppo.

I dati indicano che negli embrioni, già dai primi stadi di sviluppo i due isoenzimi sono diffusamente presenti nell'epitelio otocistico e succesivamente nell'epitelio sensoriale delle creste ampollari e delle macule acustiche, come a livello delle cellule scure del vestibolo e del *tegmentum vasculosum*. Sono, inoltre, positivi alla reazione immunocitochimica per i due isoenzimi anche l'epitelio del sacco endolinfatico e dei plessi corioidei.

Nei pulcini di un giorno e di una settimana la localizzazione dell'enzima è sovrapponibile a quella degli embrioni in cui è presente un buon differenziamento del labirinto membranoso. Gli Autori correlano tali reperti con l'azione dell'enzima nella morfogenesi e nel riassorbimento degli otoliti e con l'intervento dello stesso nel flusso di ioni e di fluidi dell'endolinfa.

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Introduction

Carbonic anhydrase (CA) catalyses the reversible reaction between carbon dioxide and water to form carbonic acid which spontaneously dissociates into bicarbonate and hydrogen ions.

CA is known to be involved in several cellular functions, such as CO_2 removal from red blood cells, acidification of secreta in the parietal cells of the gastric mucosa, selective release of K^+ and resorption of NA^+ and water in the kidney tubules. CA is also responsible for the production of aqueous humor in the eye ciliary bodies, of cerebrospinal fluid in the *choroid plexus*, and of endolymph in the *stria vascularis* of the inner ear (Watanabe, 1963; Lim *et al.*, 1983; Hsu & Nomura, 1985).

In addition, there is convincing evidence that CA may be involved in the deposition of calcium carbonate in the shell of molluscs (Wilbur & Jodrey, 1955) and chick eggs (Hodges & Lörcher, 1967), and, as regards the membranous labyrinth, in otoconia formation in chicks (de Vincentiis & Marmo, 1968; Fermin and Igarashi, 1986), mice (Purichia & Erway, 1972), and *Bufo bufo* tadpoles (Campantico, 1968).

Cytochemical CA localization in inner ear tissues was studied in the guinea pig and the rat by Watanabe (1963), the chick embryo by Marmo (1966), the chinchilla by Lim *et al.* (1983), the guinea pig by Hsu and Nomura (1985) and Takumida *et al.* (1989a).

Biochemical investigations by Erulkar and Maren (1961) in the cat inner ear demonstrated that the highest CA concentration is in the cochlear duct; moreover, Drescher (1977) found CA similar to that from red blood cells in the membranous labyrinth of mammals.

So far 7 isozymes of CA have been separated (Kaunisto *et al.*, 1990). They can be localized individually by the immunocytochemical approach, which is much more sensitive and specific than the cytochemical techniques currently used, in particular those aimed at detecting CA, which are constantly subject to criticism. Therefore, we availed ourselves of this technique, using polyclonal antibodies to localize CA I and CA II isozymes in the chick membranous labyrinth during development, which had already been investigated cytochemically by Marmo (1966). We also tried to elucidate their role in cells with different specialized functions, and in the differentiation of the inner ear structures mostly occurring during embryo development.

MATERIAL AND METHODS

Chick embryos at 2, 5, 7, 9, 11, 13 days of incubation (stage 12-39, according to Lillie, 1952), as well as 1 day and 1 week old chicks were used. The embryos were removed in normal saline, fixed in Carnoy and embedded in paraffin. The membranous labyrinths were taken from chicks after anaesthesia and decapitation, and subjected to the same procedure. In

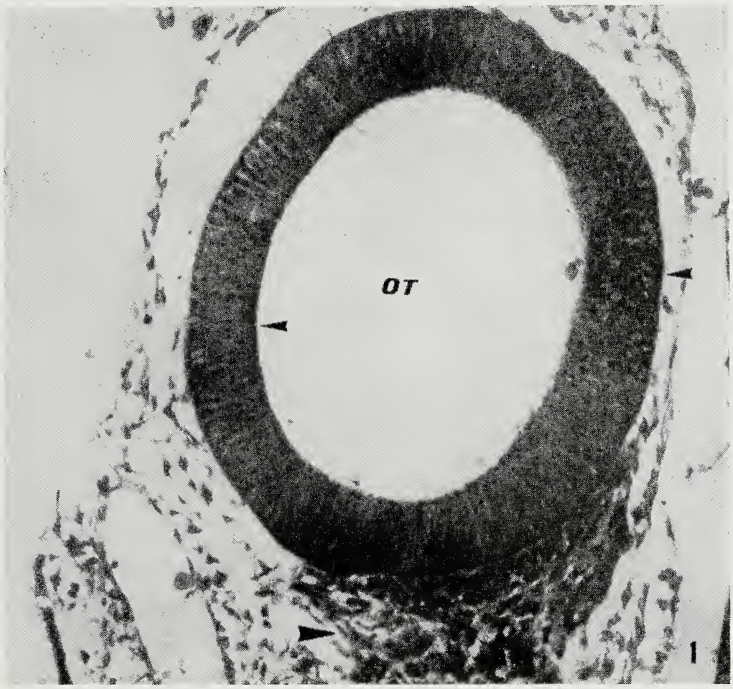

FIGURE 1 – Three day chick embryo. Diffuse positivity can be observed in the otocyst (OT) (small arrow) and in the epithelium cells of the acoustic ganglion bud (AG) (large arrow). PAP CAI. ×144

order to localize CA, the direct, indirect and peroxidase-antiperoxidase (PAP) methods were used. Deparaffined and rehydrated 5μm sections were incubated in 3% H_2O_2 for 5 min in order to inactivate endogenous peroxidase, and then pretreated with normal rabbit serum (1:20) for 30 min to block the fragment crystallizable (Fc) receptors of immunoglobulines.

The polyclonal antibodies (Serotec, Oxford, England) used were: peroxidase labelled and unlabelled sheep anti-human CA I and CA II, and

peroxidase labelled and unlabelled rabbit anti-sheep IgG; the sera were diluted 1:50 (CA I, CA II, IgG) 1:40 (PAP) in phosphate buffer saline (PBS) 0.1M pH 7.2, with 1% bovine serum albumin (BSA).

Each step was carried out in a moist chamber at room temperature for 1h. In order to reveal peroxidase, sections were then incubated in 3,3'-diaminobenzidine tetrahydrochloride (9 mg DAB in 15 ml PBS with 30% H_2O_2), and dehydrated and mounted in Canada balsam.

At the same time, negative and positive control procedures were performed by omitting the primary antibody on the sample sections, and by using guineapig kidney sections, respectively.

Results

Despite the different methods used, our investigation provided consistent data. They demonstrated that the two isozymes, CA I and CA II, are localized in the same cells; however, a quantitative study was not carried out, and hence it was not possible to establish which is the more abundant in the various sites.

Figure 2 – Five day chick embryo. It can be seen diffuse positivity of the otocyst (OT) epithelium. Endolymphatic sac (ES). PAP CA II. ×60.

In embryos at the otic pit stage, a diffuse positivity was observed in the epithelium, which persisted in the next stages until the 9th day (Fig 1 and 2). This positivity was also present in the cell cytoplasm of the acoustic ganglion bud.

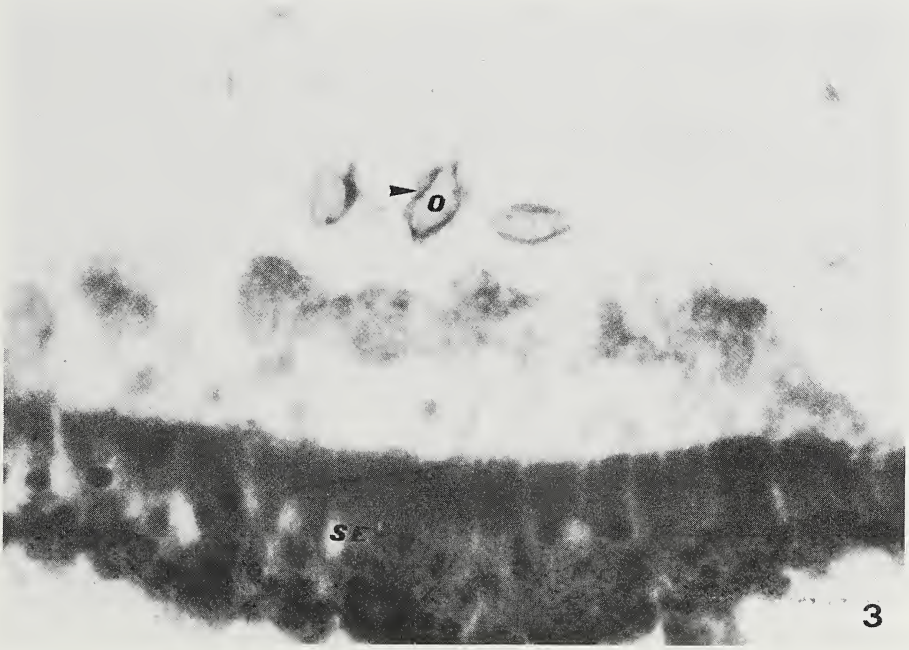

FIGURE 3 – One day chick: intensely stained sensorial epithelium (SE) of the utricle. The otolith matrix (small arrow) appears positive. Only one otolith is indicated (O). PAP CA II.×560

From the 11th day the enzyme was mostly localized in the sensorial areas of the saccule, the utricle and the lagenae, where sensorial, supporiting, transitional and dark cells showed positivity to the immunocytochemical reaction. Positivity had also been observed in the otoconial matrix since the early formation of otoconial crystals in the 7 day embryo (Fig. 3). The same distribution of the enzyme was also evident in the ampullar crests (Fig. 4). The localization of the enzyme in the epithelial cells of the endolymphatic duct and sac was already possible at early stages of development (5 days). Intense immunostaining was visualized in the dark cells of *tegmentum vasculosum* (Fig.5) and in the supporting and sensorial cells of the Corti organ.

Immunocytochemistry also evidenced abundant carbonic anhydrase in the neuron cytoplasm of cochlear and vestibular ganglia at each developmental stage (Fig. 6). In addition, a particularly intense CA reaction

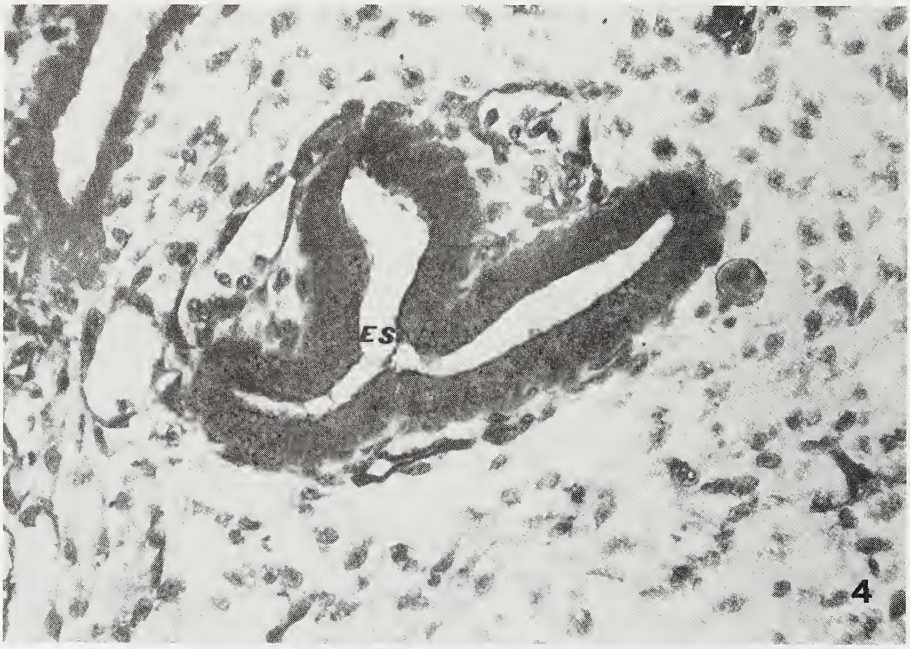

FIGURE 4 – Five day chick embryo. Intense positivity can be observed in the endolymphatic sac (ES) epithelium. PAP CA II. ×225

was also present in the epithelium of the *choroid plexus*, which are already known to show a high CA content (Fig. 7)

DISCUSSION AND CONCLUSION

Our observations showed that CA I and CA II are diffusely localized in the membranous labyrinth bud during the early developmental stages; in later stages, instead, they are restricted to the areas where they fulfil diverse and specialized functions.

CA is present in the areas (maculae) implied in otoconia production. The morphogenetic function of the enzyme is demonstrated by both the inhibition of otoconia morphogenesis, which has been observed in embryos treated with carbonic anhydrase inhibitors (Marmo, 1965; Campanti-

co, 1968; Purichia & Erway, 1972; Kido *et al.*, 1991), and by the role that the enzyme plays in crystal resorption (Lim, 1983; Kawamata *et al.*, 1986; Igarashi, 1989). Ultrastructural investigations aimed at localizing CA in the

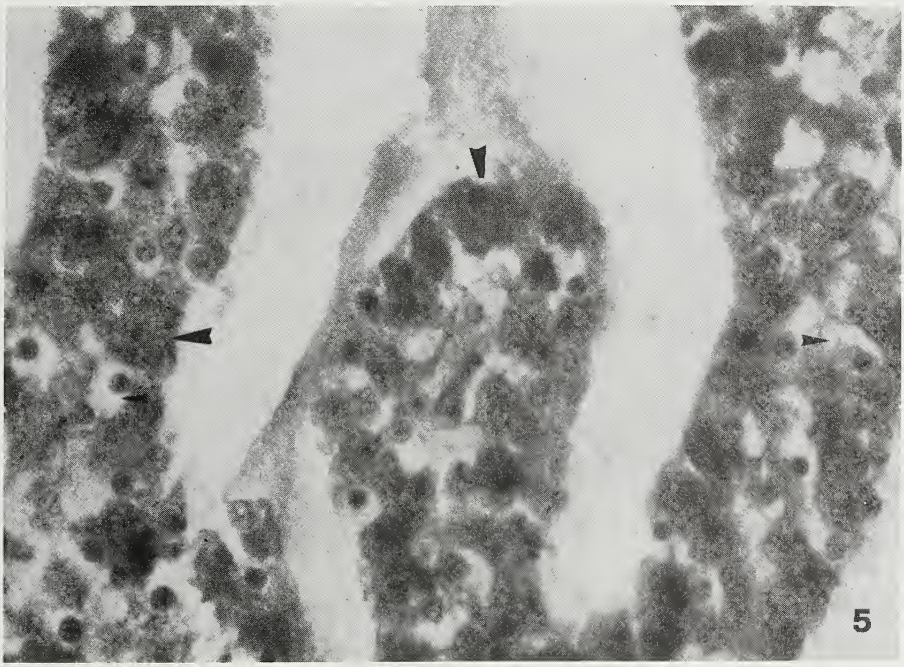

FIGURE 5 – One day chick: *tegmentum vasculosum*. It is possible to distinguish between negative light cells (small arrow) and intensely positive dark cells (large arrow). PAP CA II.×144

vestibular organ of the guinea pig have confirmed this function; in fact, they have demonstrated that the globular structures (considered otoconia precursors) that are present in the supporting, transitional and "planum semilunatum" cells are surrounded by reaction products (Takumida *et al.*, 1989 a,b). The morphogenetic role of CA in these sites is also supported by the occurrence of the enzyme in the otoconial matrix which is already present in the embryo on the early formation of otolithic crystals.

It is noteworthy that CA occurs also in cells involved in fluid and ion transportation, such as vestibular dark cells and the epithelial cells of the endolympatic sac and of the *coroid plexus*; an intense activity of Na^+ K^+ dependent ATPase and adenylate cyclase has also been demonstrated in these cells (Hsu & Nomura, 1985). However, CA is also present in cells not

being involved in fluid and ion transportation, such as supporting cells, that, as suggested by Lim (1983), may play an important role in the respiratory mechanism of sensorial cells.

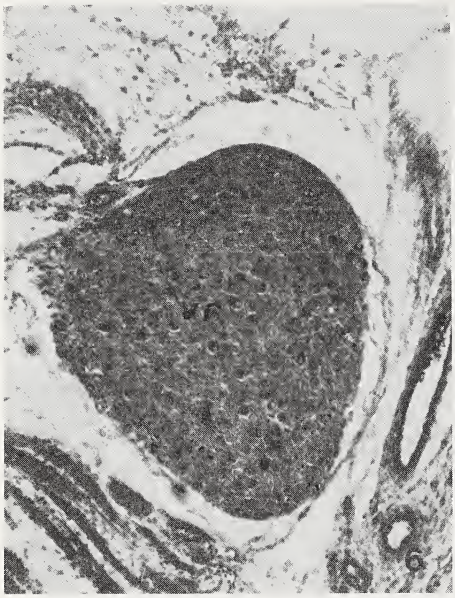

FIGURE 6 – Thirteen day chick embryo: vestibular ganglion (VG). Positivity is evident in the neuron cytoplasm (small arrow). PAP CA I.×60

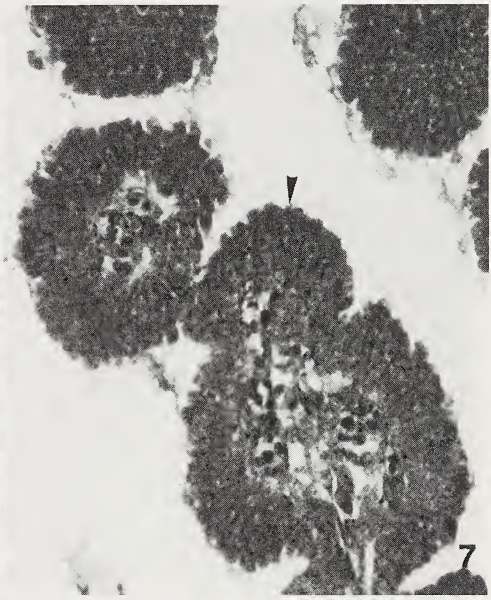

FIGURE 7 – Thirteen day chick embryo: choroid plexsuses. Positivity is clearly evident in the epithelium (small arrow). PAP CA II×225

Interestingly, unlike the cytochemical data obtained by Lim *et al.* (1983) and Hsu and Nomura (1985) in the maculae, the cristae and the Corti organ, our investigation evidenced an intense CA reaction not only in supporting cells, but also in the sensorial ones. As regards more specifically the chick embryo membranous labyrinth, our data showed that the enzyme fails to disappear in 15 day embryos, in contrast to what was demonstrated in previous cytochemical studies (Marmo *et al.*, 1966), but persists after hatching showing the same localization as in the embryo at advanced stages of development.

In conclusion, the present study does not provide any clue to the possible role played by CA in the inner ear. Here CA activity is different in

the various cell types ("secretory", supporting and sensorial cells) , and hence carbonic anhydrase might be a ubiquitous enzyme. CA might be involved in specialized functions only in the cells showing intense enzymatic activity. Therefore, it could be suggested that this enzyme may play several functions in the inner ear: i) regulation of the endolymph ions and/or fluids; ii) removal of CO_2 from inner ear tissues; iii) otoconia formation and maintenance; iv) influence on the endolymphatic potential, in agreement with Prazma (1978) and Vozumi *et al.* (1991), as well as on the neural stimulus spread, in view of its occurrence in the cytoplasm of acoustic ganglion neurons, as already suggested by other investigators (Giacobini, 1961; Korhonen *et al.*,1964; Korhonen & Korhonen, 1965).

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Ocurrence of β-endorphin- and enkephalin- like immunoreactivity in the hypothalamus of the domestic fowls, *Gallus domesticus*

Esposito V., De Girolamo P., Sammarco M., Gargiulo G.

Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università degli Studi «Federico II», Via Delpino, 1, I-80137 Napoli, Italy

Key words: Immunocytochemistry, hypothalamus, enkephalins, β-endorphin, *Gallus domesticus*.

Abstract. The distribution of β-endorphin and enkephalin-like immunoreactivity in the hypothalamus of 2-week-old domestic chicks was studied with immunocytochemical methods, using antibodies to β-endorphin, met-enkephalin and leu-enkephalin. β-Endorphin-like neurons were observed in the nucleus infundibuli whereas β-endorphin-like fibers were detected both in the infundibular area and in the external layer of median eminence. Enkephalin-like immunoreactive perikarya were observed in the parvocellular division of the nucleus paraventricularis and in the nucleus infundibuli. The enkephalin-like immunoreactive fibers were observed in every part of the hypothalamus, but mainly concentrated in the infundibular area and in the external layer of median eminence. The distribution pattern of both β-endorphin and enkephalins is compared with that other neuropeptides present in the chick hypothalamus. The results are discussed in relation to the possibility that both β-endorphin and enkephalins may be involved in hypophysial regulation or neuromodulator activity in the hypothalamus of domestic chick.

Riassunto. Presenza di immunoreattività β-endorfina ed encefalino-simile nell'ipotalamo di *Gallus domesticus*.

Nel presente studio immunocitochimico eseguito sulla regione ipotalamica di pulcini di Gallus domesticus secondo il metodo di perossidasi antiperossidasi, ha evidenziato la presenza di immunoreattività ad anticorpi per la metionina-encefalina, leucina-encefalina e β-endorfina. Test di specificità hanno dimostrato "cross"-reattività tra i due antisieri per le encefaline ed i rispettivi antigeni e tra l'antisiero per la β-endorfina e la β-lipotropina, per cui l'immunoreattività appresso descritta è da considerarsi relativa a sostanze encefalinosimile e β-endorfina e/o β-lipotropinasimile.

L'immunoreattività all'anti-β-endorfina è stata messa in evidenza in corpi cellulari presenti nella regione del nucleo infundibulare ed in fibre principalmente frammiste alle cellule suddette; essa risulta anche presente, seppur scarsamente, nella regione ipotalamica anteriore e nella zona esterna dell'eminenza mediana anteriore e posteriore.

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L'immunoreattività encefalino-simile è presente in neuroni appartenenti alla componente parvocellulare del nucleo paraventricolare ed in cellule del nucleo infundibulare; ed inoltre, in fibre sparse in tutto l'ipotalamo e nella zona esterna dell'eminenza mediana anteriore e posteriore.

La presente localizzazione rapportata a quella di altri sistemi peptidergici ipotalamici, già caratterizzati in lavori precedenti, evidenzia correlazioni spaziali il cui significato funzionale è da stabilire, ma che suggeriscono complessi rapporti neuromodulatori soprattutto nella regione infundibulare. La presenza di immunoreattività encefalino- e β-endorfino-simile nella zona esterna dell'eminenza mediana depone a favore dell'ipotesi che questi peptidi siano rilasciati nella circolazione portale ipofisaria e quindi, coinvolti nella funzione neuroendocrina del sistema ipotalamoipofisario.

Introduction

Both methionine-enkephalin (met-ENK) and leucine-enkephaline (leu-ENK) as well as β-endorphin (β-END), are all members of a natural opioid peptide family (Hughes *et al.*, 1980) able to link endogenous opiate receptors in the brain (Terenius & Wahlstrom, 1974; Hughes, 1975) and in the pituitary gland of various vertebrates (Cox *et al.*, 1975).

At first, β-END was isolated and characterized from camel and porcine pituitaries as a 31amino-acid peptide, identical to the carboxyl terminal sequence of β-lipotropin (nLPH) which is considered its biosynthetic precursor (Brandbury *et al.*, 1976; Li & Chung, 1976; Chretien *et al.*, 1976; Cox *et al.*, 1976; Akil *et al.*, 1984).

Both met-ENK and leu-ENK were isolated and characterized from the pig brain as pentapeptides (Hughes *et al.*, 1975) and they stem from a large precursor molecule, called proenkephalin (Akil *et al.*, 1984).
In mammals the enkephalin-containing neurons are distributed widely in the central nervous system, both as local circuit neurons and as projection neurons, whereas β-END-containing neurons are mainly concentrated in the region of the arcuate nucleus of the mediobasal hypothalamus with fibers projecting rostrally and caudally to many limbic, thalamic and lower brainstem targets; although, another cell cluster is present in the *nucleus tractus solitarius* (Elde *et al.*, 1976; Hökfelt *et al.*, 1977; Simantov *et al.*, 1977; Sar *et al.*, 1978, Bloom *et al.*, 1978; Uhl *et al.*, 1979; Bugnon *et al.*, 1979; Bloch *et al.*, 1979; Sofroniew, 1979; Pickel *et al.*, 1980; Finley *et al.* 1981; Schwartzberg & Nakane, 1981; Khachaturian *et al.*, 1985 a,b).

The very few observations on the distribution of opioid peptide in the avian brain, include mainly the enkephalins (Bälhser & Dubois, 1980; De Lanerolle *et al.*, 1981; Ryan *et al.*, 1981; Mikami *et al.*, 1983; Ball *et al.*, 1988).

Further to our previous studies on characterization of hypothalamic peptidergic systems in the chick brain (Gargiulo *et al.*, 1990; Esposito *et al.*, 1992 a,b), we have identified in the present investigation, by immunocytochemical methods, enkephalin and β-endorphin-like centers in the hypothalamus of the chick *Gallus domesticus*. The probable interaction with other hypothalamic neuropeptides and their involvement in regulation of the release of pituitary hormones have been discussed.

Materials and methods

Male chicks of *Gallus domesticus* were obtained from a local breeder at the age of two weeks. Chicks were killed by decapitation and their brains were rapidly removed and immersed in Bouin's fixative solution. The fixed brains were then routinely dehydrated through a series of graded alcohols, placed in xylene, and embedded in paraffin. Sagittal and transverse serial sections of the brains 5μm thick, were cut and mounted on glycerinealbumin coated glass slides. Finally, sections were deparaffinized in xylene, rehydrated in a graded ethanol series and washed in 0.01M phosphatebuffered saline (PBS), pH 7.4.

The immunocytochemical staining was performed using the peroxidaseantiperoxidase (PAP) technique (Sternberger, 1979).

Briefly, after inhibition of endogenous peroxidase activity with 3% hydrogen peroxide, the sections were treated with normal goat serum (IgG, 1:5, 30 min.), to avoid possible background reaction; then incubated overnight at 4°C with antisera to met-ENK diluted 1:500/1500, leu-ENK diluted 1:500/1500 and β-END diluted 1:1000/2000. Subsequently, the sections were treated with goat antirabbit immunoglobulin (1:50, 30min.) and PAP-complex (1:100, 30min.). The reaction was visualized with a solution of 3,3'-diaminobenzidine (DAB; 10 mg in 15 ml of 0.5 M Tris-buffer, pH 7.6 containing 1.5 ml hydrogen peroxide at 0.03%). Between each step, the sections were thoroughly rinsed in PBS.

Antisera to β-END (i456/002), met-ENK (i672/002) and leu-ENK (i671/002) was purchased from UCB as well as β-END (VB056), met-ENK (VB051), leu-ENK (VB050), normal goat serum (i200/002), goat anti-rabbit serum (i200/003) and PAP-complex (i200/001). DAB was purchased from DAKO.

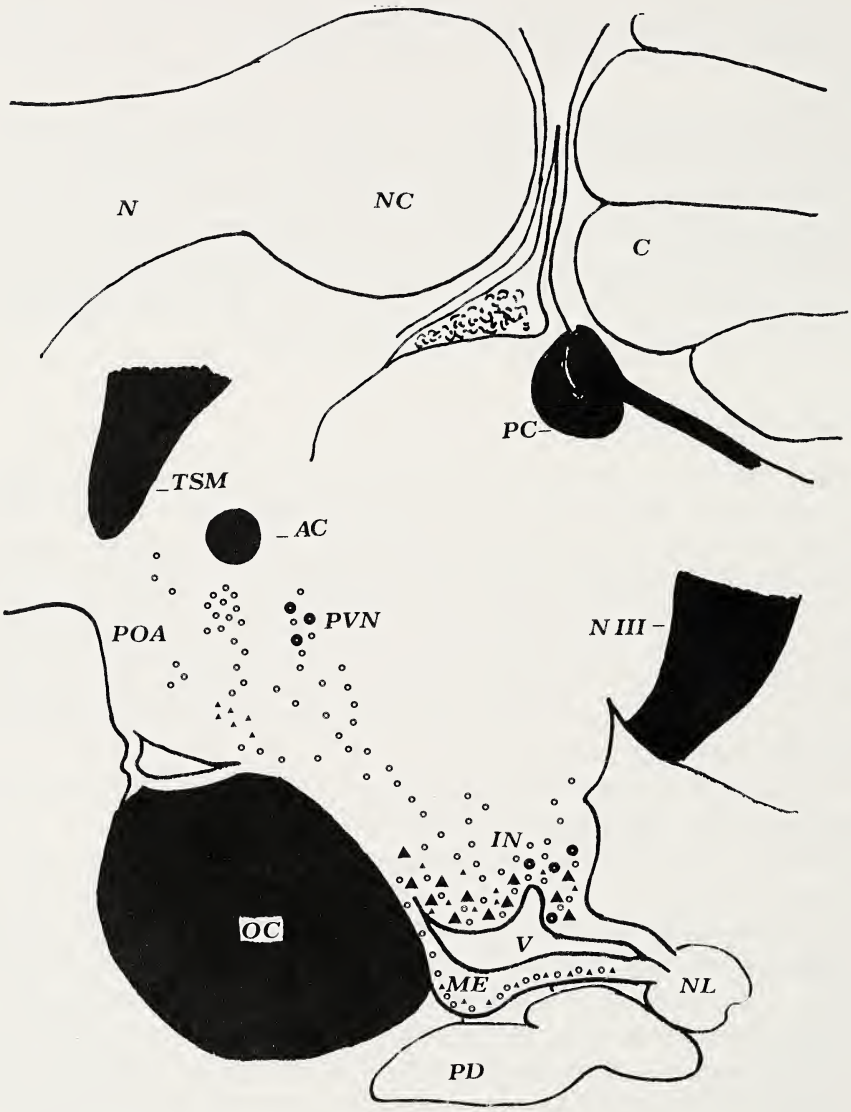

FIGURE 1 – Schematic drawing of the hypothalamus of domestic chick in a parasagittal plane, showing the distribution of the immunoreactive perikarya and fibers to antiβendorphin (▲, ▲) and to anti-enkephalins (●, ●). AC: anterior commissure; C: cerebellum; IN: nucleus infundibuli; ME: median eminence; N: neostriatum; NC: neostriatum caudale; NL: neural lobe; NIII: nervus oculomotorius; OC: chiasma opticum; PC: posterior commissure; PD: pars distalis; POA: preoptic area; PVN: nucleus paraventricularis; TSM: tractus septomesencephalicus; V: third ventricle.

The specificity of staining were verified with control experiments as follow: a) replacement of the metENK antiserum with the same antisera adsorbed by both enkephalins, b) replacement of the leu-ENK antiserum with the same antisera adsorbed by both enkephalins; c) replacement of the β-END antiserum with the same antisera adsorbed by β-END, β-LPH and both enkephalins, d) replacement of the met-ENK, leu-ENK, and β-END-antisera with normal rabbit serum or phosphate-buffered saline.

Some sections were Luxol fast bleu-cresyl violet stained to improve the identification of nervous structures.

All the sections were dehydrated, coverslipped and finally observed and photographed using a Leitz Aristoplan.

Results

Immunocytochemical reactions with the β-END, met-ENK and leu-ENK-antisera were observed in different parts of the hypothalamus of the domestic chick, *Gallus domesticus* (Fig. 1).

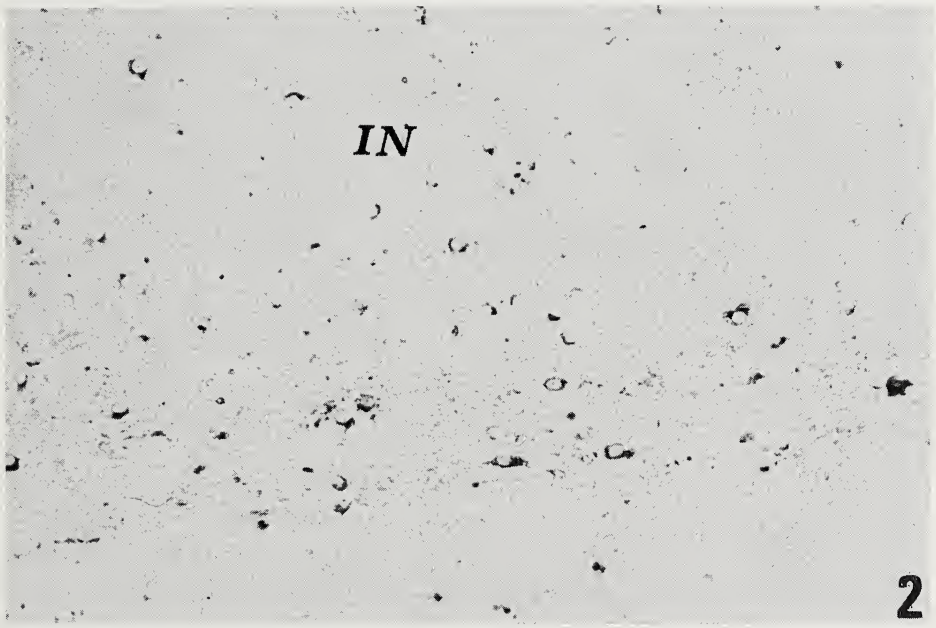

Figure 2 – Photograph showing β-endorphin-immunoreactive cells and fibers in the n. infundibuli. Parasagittal section.(X350). IN: n. infundibuli.

Immunocytochemical specificity controls were carried out on adjacent serial sections in each area where positive immunoreactivity to β-END, met-ENK and leu-ENK were identified. The absorption tests by both synthetic methionine-enkephalin and leucine-enkephalin showed that each studied enkephalin antisera revealed both homologous antigens and heterologous antigens; indeed, it was observed no immunoreactivity or attenuated staining when preabsorption of met-ENK antiserum was carried out with synthetic met-ENK or leu-ENK, on the other hand the preabsorption of leu-ENK antiserum with synthetic leu-ENK or met-ENK inhibited or attenuated respectively the immunostaining.

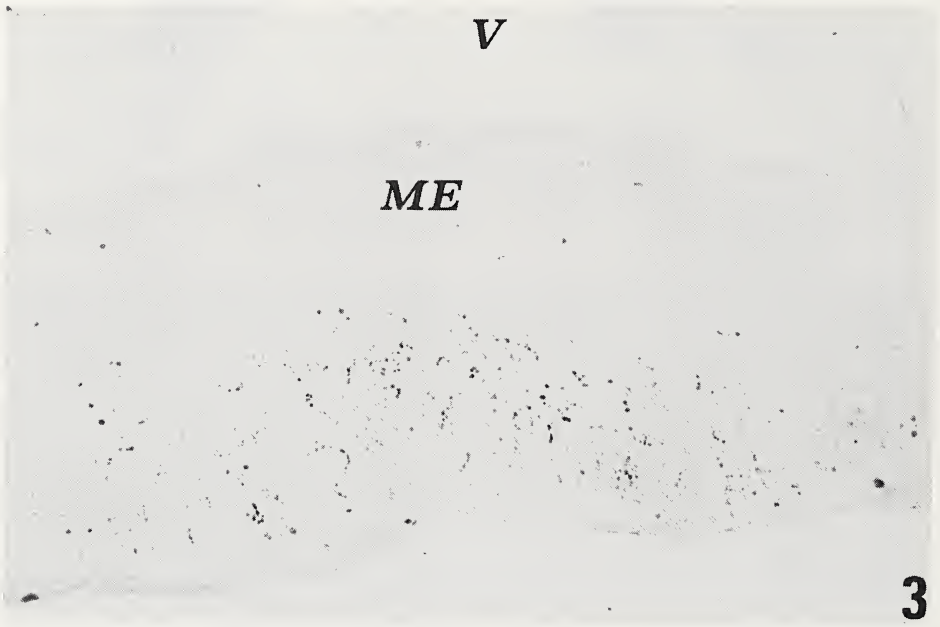

FIGURE 3 – Photograph showing β-endorphin-immunoreactive fibers in the zona externa of median eminence. Transverse section. (X300). ME: median eminence, V: third ventricle

The β-END immunostaining was inhibited by absorption of the β-END antiserum with both synthetic β-END and β-LPH; thus, the employed antiserum can recognize both β-END and β-LPH. The β-END immunostaining was maintained when it was used the β-END antiserum adsorbed by both synthetic met-ENK and leu-ENK.

No immunoreactive reaction was detected when control slides were treated with normal rabbit serum or with phosphate-buffered saline instead of the specific antiserum.

For the identification of the anatomical structure, and the following nomenclature we referred to Kuenzel and van Tienhoven (1982).

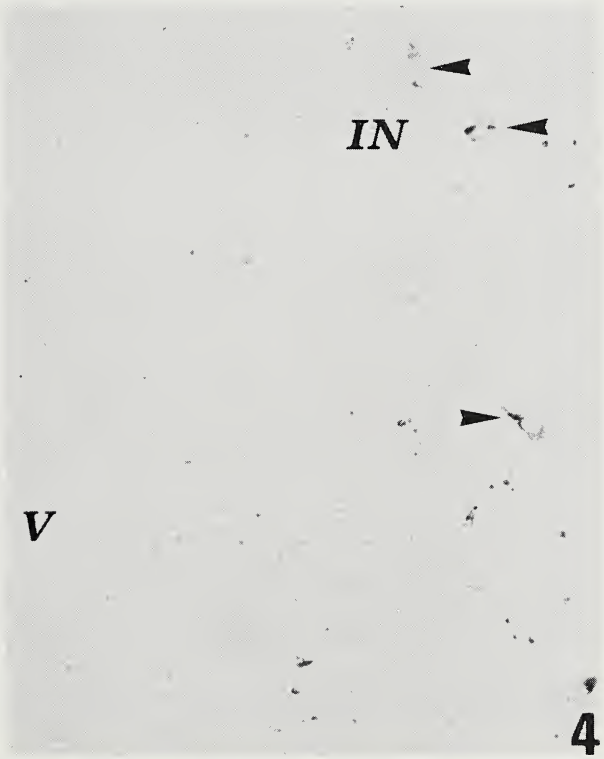

FIGURE 4 – Photograph showing β-endorphin-immunoreactive cells (arrows) in the n. infundibuli. Transverse section. (X400). IN: n. infundibuli: third ventricle

A large population of β-ENDlike immunopositive cell bodies occurred in the nucleus infundibuli, a neuron group located ventromedial to the third ventricle (Figures 2 and 4).

β-END-like immunopositive fibers were detected in the mediobasal hypothalamus and in the nucleus infundibuli intermingled with β-END-immunopositive perikarya. Only few thin β-END immunoreactive-fibers were found scattered in the anterior hypothalamic area. Thin β-END-like immunopositive fibers were also distributed in the zona externa of the median eminence (Fig. 3).

Enkephalin-immunoreactive neurons were found both in the parvocellular division of the nucleus paraventricularis and in the nucleus infundibuli; the later were very few and small in size (Figures 5 and 6).

ENK-immunopositive fibers were detected throughout the whole hypothalamus; anyhow they were mainly concentrated in the anterior hy-

FIGURE 5 – Photograph showing enkephalin-immunoreactive cells (arrow) and fibers in the median eminence. Transverse section. (X250). IN: n. infundibuli, ME: median eminence

pothalamus, ventrally to the anterior commissure (Fig. 8), and in the posterior hypothalamic region around the noimmunoreactive perikarya of the nucleus mammillaris medialis (Fig. 7).

DISCUSSION

The findings of present immunocytochemical study indicate that both enkephalinergic and β-endorphinergic or lipotropinergic system are pre-

sent in the chick hypothalamus and that they have a different localization as well as mammals (Bloom *et al.*, 1978; Watson *et al.*, 1978).

Specificity tests for immunocytochemical staining revealed a possible cross-reaction between the anti-MET-ENK antisera and LEU-ENK, and the anti-LEU-ENK antiserum and MET-ENK; the present study, therefore, reports the localization of an enkephalin-related peptide.

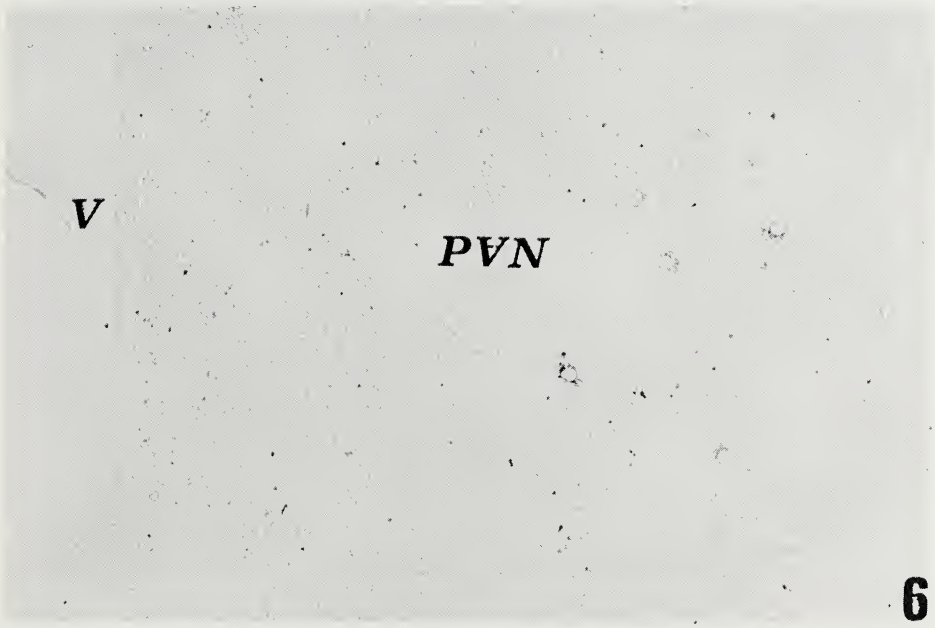

FIGURE 6 – Photograph showing enkephalinimmunoreactive cells in the n. paraventricularis. Transverse section. (X300). PVN: n. paraventricularis, V: third ventricle

On the other hand, the anti-human β-endorphin antiserum employed in this study reveal cross-reactivity with β-lipotropin, suggesting that they also contain antigenic determinants common to β-LPH. Similar behavior has been reported previously for antihuman and anti-porcine β-END (Bloch *et al.*, 1979; Doerr-Schott *et al.*, 1981; Kiss *et al.*, 1985).

Other immunocytochemical studies on lower vertebrates (Doerr-Schott *et al.*, 1981; Nozaki & Gorbman, 1984; Vallarino, 1985, 1986) indicate that cross reactions may occur between mammal anti-β-END and the corresponding antigen from nonmammalian vertebrates, suggesting an immunogenic relationship between β-END molecules among vertebrates.

The β-endorphin-like immunoreactivity evidenced in the present study is mainly concentrated in the nucleus infundibuli that appears to be the homologous of the arcuate nucleus of mammals (Mikami, 1986), the principal site of β-END-containing neurons in mammalian species (Akil *et al.*, 1984).

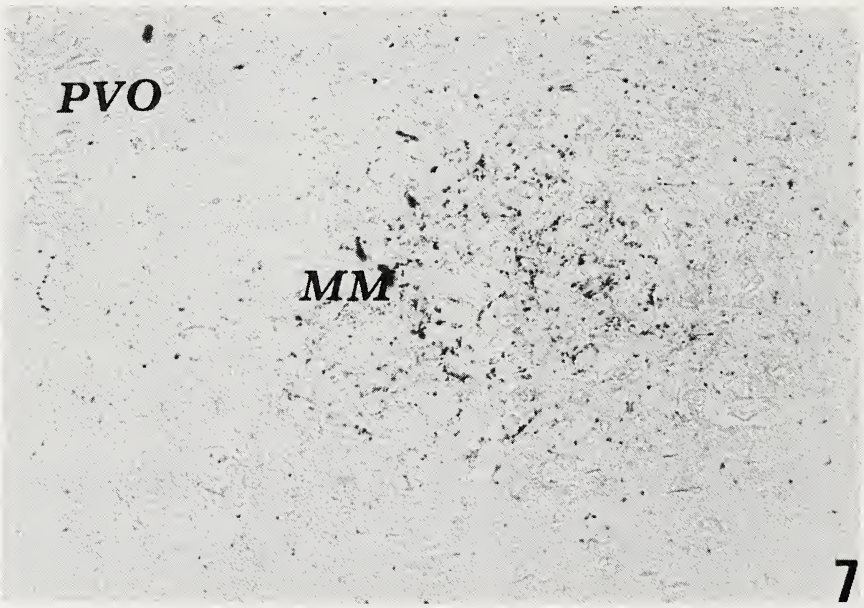

FIGURE 7 – Photograph showing enkephalin-immunoreactive fibers in the n. mammillaris. To remarke the network fibers around the noimmunopositive perikarya of MM. Transverse section. (X300). MM: n. mammillaris, PVO: paraventricular organ

Many kinds of neuropeptides have been immunocytochemically characterized in the avian nucleus infundibuli (Mikami, 1986; Esposito, 1990; Gargiulo *et al.*, 1990; de Girolamo & Esposito, 1991; Esposito *et al.*, 1992 a, b); the physiological significance of this spatial relationship between β-END and other peptides is unknown, but it is possible to postulate complex neuromodulator interaction. Furthermore, the β-END-immunoreactive fibers terminals in the median eminence coming very likely from nucleus infundibuli suggest a possible hypophysiotropic role of β-END. Sakurai *et al.* (1986) demonstrated that β-END is involved in the control of LH release in the hen as an inhibitory agent.

According to Blähser and Dubois (1980) we have observed that immunocytochemical reaction for enkephalins reveals more extensive fiber network than immunoreactive perikarya confirming the possibility of a discontinuous peptide synthesis within the perikarya, or an unmasking of the peptide molecule only within the axons and dendrites.

FIGURE 8 – Photograph showing enkephalinimmunoreactive fibers and some cells (arrows) in the anterior hypothalamus. Parasagittal section. (X350)

However the hypothalamic distribution of immunoreactivity to enkephalins described in this study is similar to those reported in previous works (for review see Viglietti-Panzica & Panzica, 1991).

The enkephalinergic centers observed in the hypothalamic region, in both paraventricular and infundibular perikarya, may be related to the presence in this area of a wide neurosecretory system producing hypophysiotropic hormones (Scharrer & Scharrer, 1963; Blähser, 1983). Generally, the ENK appear to inhibit the neuronal firing rate by inhibiting transmitter-induced depolarization of the postsynaptic cell membrane (Zieglgansberger *et al.*, 1976, North & Williams, 1985), therefore they may modulate different excitatory signals in this area or in other hypothalamic sites.

The present reports mainly emphasizes the rich enkephalinergic innervation of the infundibular area which is consistent with the various hypophysiotropic effects of enkephalins (Tramu *et al.*, 1981).

The presence of perivascular enkephalins fiber terminals in the median eminence implies that enkephalin may be released from the median eminence into the portal circulation and involved in neuroendocrine function of the hypothalamo-hypophysial system.

Finally, our observation show a spatial relationship between ENK and β-END in the mediobasal hypothalamic area, which contains the main accumulation of β-END perykaria, intermingled with ENK-fibers, but the possible interaction between them will be able to demonstrate only at the ultrastructural level.

In conclusion, these observation indicate the presence of both enkephalin-like and β-endorphin-like immunoreactive substance in the chicken hypothalamus, which might be involved in the pituitary regulation and/or in other neuronal functions as neuromodulator.

This study also provide a foundation for further studies to elucidate the role of β-END in the brain of the domestic chick and other birds.

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Action of the cadmium on the *Carassius auratus* living in lentic waters containing catabolic ammonia

Battaglini P.[1], Soppelsa O.[1], Improta C.[2], Ferrara L.[2]

[1] Dipartimento di Zoologia, Università degli Studi «Federico II», via Mezzocannone 8, 80126 Napoli, Italy

[2] Dipartimento di Chimica, Università degli Studi «Federico II», via Mezzocannone 4, 80126 Napoli, Italy

Key words: cadmium, ammonia, water hardness, *Carassius auratus*.

Abstract. In a previous paper (Battaglini *et al.*, 1991) concerning the Cadmium ion absorption in different *Carassius auratus* organs in "soft" and "hard" waters, the Authors have shown that the absorption of the Cadmium is much more significant in softwater than in hard water. In this paper the Authors have considered a further element characterizing the habitat of the *Carassius auratus*, i.e. the limited environment of stagnant water in which, besides the pollution substances like cadmium salts, the same catabolic materials of the *Carassius auratus* exist. The *Carassius auratus* were kept for 8 days to become acclimatized. We have then carried out a research on the results obtained by our tested animals to the Cadmium collecting the survival and absorption data of this toxic in different organs by adding cadmium chloride $CdCl_2.2^1/_2H_2O$ up to reach a 10 ppm concentration of ion Cd^{++} in experiment tanks where. We have observed that the storage in water of catabolic material such as ammonia is an element increasing cadmium toxicity related to the *Carassius auratus*. Such toxicity is revealed both by higher values of absorption of this ion in different organs and in the survival values of the specimens which are definitely lower.

Riassunto. Azione del cadmio su *Carassius auratus* viventi in acque lentiche contenenti NH_3 catabolica

In un precedente studio relativo all'assorbimento dello ione cadmio nei vari organi di *Carassius auratus* in acque "dure" e "dolci", abbiamo dimostrato che l'assorbimento del cadmio è molto più pronunciato in acqua dolce che in quella dura. Inoltre, avendo constatato in acqua dura contrariamente a quanto avviene in acqua dolce una immediata e quasi completa precipitazione dello ione cadmio sottoforma di $CdCO_3$, abbiamo ipotizzato nei due casi due differenti meccanismi di assimilazione del cadmio: a) per osmosi con bioaccumulo nel tempo, in acqua dolce; b) per ingestione di $CdCO_3$ solido precipitato, nel caso di acqua dura.

Nel presente lavoro abbiamo voluto prendere in considerazione un ulteriore elemento che caratterizza l'habitat del *Carassius auratus*, ovvero l'ambiente limitato di acqua ferma in cui sono presenti, oltre ad eventuali sostanze inquinanti come i sali di cadmio, gli stessi prodotti di rifiuto dei carassi. Abbiamo quindi

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effettuato uno studio sulla risposta fornita dai nostri animali da esperimento al cadmio, raccogliendo dati di sopravvivenza e di assorbimento di tale tossico nei vari organi. In particolare abbiamo aggiunto cadmio cloruro, $CdCI_2.2^1/_2H_2O$, fino a raggiungere una concentrazione di 10 ppm di ione Cd^{2+} in vasche da esperimento ove erano stati tenuti i carassi per acclimatarli nei precedenti 8 giorni. Nell'acqua utilizzata, ("acqua dura": pH=7,8; durezza 369 mg/L di $CaCO_3$; "acqua dolce" pH=7,6; durezza 152 mg/L di $CaCO_3$) erano presenti anche i prodotti catabolici dei carassi tra cui l'ammoniaca che, al momento della aggiunta del cadmio, presentava una concentrazione di 7.10 mg/L. Le misure di assorbimento atomico sono state eseguite sugli organi mineralizzati. I pesci sono stati sacrificati ai seguenti tempi: 1h 30', 24h, 72h, 7gg. La presenza nell'acqua di NH_3, che è un forte complessante dello ione cadmio, ha notevolmente aumentato la solubilità dei suoi sali permettendo la sua permanenza in soluzione al 100% per oltre 4 giorni. Successivamente lo ione metallico è cominciato a precipitare come carbonato, rimanendo tuttavia per circa il 20% del suo valore iniziale ancora al 7° giorno dall'inizio dell'esperimento. La sopravvivenza nella "acqua dura" è stata del 100% dopo 1h 30', del 50% dopo 24h, del 44% dopo 72h e del 12% dopo 7gg. Nella "acqua dolce" la sopravvivenza, molto simile alla precedente, è stata del 100% dopo 1h 30', del 50% dopo 24h, del 40% dopo 72h e del 10% dopo 7gg. I dati di assorbimento confermano che gli organi che presentano maggiore accumulo di cadmio sono, come nel nostro precedente esperimento, fegato, rene ed intestino, mentre si ottengono valori modesti o addirittura nulli per pelle, cervello, cuore e muscolo. Inoltre i valori di assorbimento di Cd^{2+} sono sempre più elevati rispetto a quelli riscontrati per esemplari posti in acqua senza ammoniaca. Ciò è stato attribuito al fatto che essendo il cadmio, nel caso attuale, presente in massima parte in soluzione, anche se come complesso cadmioammoniaca, esso possa essere più facilmente assimilato per assorbimento dai Ciprinidi (nel nostro caso *Carassius auratus*) e accumulato nei vari organi. In conclusione si può dire che l'accumulo nell'acqua di un prodotto catabolico come l'ammoniaca costituisce un elemento di aggravamento della tossicità del cadmio nei confronti dei carassi, che si manifesta sia in più elevati valori di assorbimento di questo ione nei vari organi che nei valori di sopravvivenza degli esemplari che sono drasticamente più bassi.

Introduction

The pollution of the waters caused by heavy metals and the relevant consequences on flora and fauna of these ecosystems has become a very topic question following the great development of human activities at industrial level. Among the different heavy metals cadmium and mercury proved to be the most toxic, (Ravera, 1984). This metal which is present in nature expecially as an impurity of the zinc minerals (blenda, ZnS) or as sulphur (grenockite CdS), is spread out in the biosphere through different ways such as: volcanic activity, exudates from vegetation, forest fires, windblown dust and leaching of rocks and, in the latest ten years, also

through the use of phosphate fertilizers, incinerator waste coal and oil combustion and the same industrial production of cadmium minerals (Ravera, 1984; Polprasert, 1982). The cadmium, spread out in the soil, in discharge waters and in atmosphere, is at last carried by wind and rain into the streams of water (Absullah & Royle, 1972; Ajmal *et al.*, 1985; Ajmal *et al.*, 1987).

The studies on cadmium toxicity on freshwater fish have shown a significant dependence of this toxicity from the chemical characteristics of the environment in which the toxic is spread out, having sometimes increasing results, some other times decreasing results, ofthe toxic action ofthis heavy metal (Sprague, 1987; Calamari *et al.*, 1980; Enk & Mathis, 1977).

In former studies (Battaglini *et al.*, 1991; 1992a; 1992b; Gargiulo *et al.*, 1991) concerning ion cadmium uptake in different organs of *Carassius auratus* in waters of different hardness, it was shown that the metal uptake is much more significant in soft than in hard water. In addition, owing to the observation of a heavy and sometimes almost complete precipitation of ion cadmium as $CdCO_3$ in waters of higher hardness, two possible ways of absorption with consequent toxic action of cadmium were assumed: a) due to osmosis with bioaccumulation during the time (above all in soft water); b) due to intake of $CdCO_3$ solid precipitate (above all in hard water) (Battaglini *et al.*, 1991).

The high and sudden mortality of fish in rivers and hatcheries is often caused by lethal concentrations of polluting toxicant substances (Tarazona *et al.*, 1987). Anyway such pollution is not always due to the intake of allochtoneous substances, as the same catabolic materials in small habitat and without change water can cause an environmental change, being themselves toxic or simply increasing the toxicant action of other substances already present in the habitat (Ravera, 1984).

To better understand the environmental changes that are displayed on the area, some behavioural and echotoxicology tests were carried out on goldfish *(Carassius auratus)* aimed at focusing the interrelation, from an ecotoxicology view, of some behavioural parameters.

In this paper the authors have considered an element that characterizes the natural habitat of *Carassius auratus*, i.e. the feature of a limited habitat with stagnant water in which, besides the possible polluting substances, there is a storage of catabolic materials of *Carassius auratus* (Mommsen & Walsh, 1992). Among these, ammonia is the major nitrogenous and product comprising usually more than 70% of total waste nitrogen in freshwater teleosts (Warde van, 1983). The importance of

ammonia in this context is due not only to its real toxicity but also because it is one of the main chemical complexing substances of ion Cd^{2+}, causing a modification of the speciation of this metal in solution.

Thus the authors have carried out a study on *Carassius auratus* response to the polluting action of cadmium with ammonia as catabolic product, testing such action in waters of different hardness in order to compare these results with those obtained in former experiments carried out without ammonia.

Materials and methods

20 tanks of 20 l each were prepared, of which 10 tanks were filled up with tap water of Naples and 10 were filled up with the same water, previously decalcified with ion exchange resins Soft C (line "C") CARMAR, Naples. Both kinds of waters were utilised only after a period of aeration in a 600 l glass aquarium to eliminate the chlorine.

The test fish, supplied by CARMAR Co., Naples, weighed $5 \div 7$ g.
All animals have been held for 10 days to have them acclimatized in two different kinds of waters in two 600 l separate glass aquaria, equipped with air pump and filter Eheim, in the Ecology and Ecotoxicology Laboratory of the Department of Zoology University of Naples Federico II.

Then 200 *Carassius auratus*, randomly chosen, were transferred in experiment tanks (10 fishes in each tank). The tanks had a photoperiod of 12 hrs and were supplied with an air pump, but without filter, to prevent catabolic materials from being eliminated To simulate the lentic environment, fishes were held for 8 days in the same water to let catabolic materials store up, and particularly ammonia. During this period, fish were fed as usual. The experiment began only after these eight days. Cadmium as $CdCl_2.2^1/_1H_2O$, equal to 10 ppm, was added to 8 tanks with water of higher hardness and to other eight tanks with water of lower hardness. The *Carassius auratus* which were in other 4 tanks, 2 tanks with soft water and two tanks with hard water, were utilized as control fishes during the experiment. During the whole experiment the temperature was constantly at 16 ± 1°C.

The analysis of the different substances which are in the two different kinds of waters utilized for the experiment, was performed at zero time. Measures were taken utilizing the traditional volumetric titration, the standard solution hardness of sodiumtilendiaminotetracetic acid salt for the hardness, alkalinity with standard solution of HCl using bromocresol as

acidbasic indicator for the alkalinity, ionchloride with $AgNO_3$ solution utilizing Mohr (Kolthoff *et al.*, 1973) method for ionchloride. A pHmeter ORMA, model NK 300 was used to determine the pH and a dissolved oxygenmeter HI 8543 (Hanna Instruments, USA) to determine the dissolved oxygen in water.

The NH_3 produced and present in water was measured with a spectrophotometer utilizing Nessler reagent to obtain the typical yellow colour.

Cadmium concentration in solution in the single tanks was measured every 24 hrs till the end of the experiment by using the atomic absorption spectrophotometer VARIAN AA275. To get the correct interpretation of cadmium concentration values present in solution at different phases, a kinetic precipitation of ion Cd^{2+} as $CdCO_3$ was carried out in parallel in the same waters utilized during the tests, but without *Carassius auratus* and its relevant catabolic materials.

The survival values were taken up to 7 days from the beginning of the experiment, carrying out also the pathological anatomy dissection of the dead specimens.

To evidence the metal effects on *Carassius auratus* behaviour the ventilation frequency of experimental and control goldfish was determined by counting the number of opercular movements per minute (Lang *et al.*, 1987) of 5 fishes for each tank. The computation of 5 fishes for each tank was optimized by using the average value, which was then compared to the values obtained at different phases. Such computations were made every 15' for the first 90 minutes, every 30' for the following 150 minutes and every 24 hours during 3 more days. A further parameter to understand the behavioural reactivity in the presence of toxic was taken counting the movements of the caudal fin per minute (Abel & Papoutsoglou, 1987): more beats = more movement. Of course the same tests were carried out for the controls. For each kind of water two specimens were taken randomly in various periods of time (1.5 h, 24 h, 72 h and 168 h). The organs taken out, after being weighed on an analytic balance, were treated with a mixture (weighing as much as five times the organs) of $HNO_3/HClO_4$ 4:1 in a 10 ml pyrex tube plugged at 7080°C for 24 h in order to obtain a complete mineralization of the tissues. The solution obtained was brought up to the known volume by adding HNO3 0.1 M and then analyzed by an atomic absorption spectrophotometer VARIAN AA275 in order to determine the cadrnium concentration as parts per million (ppm) of fresh organ.

Results

In both experiments the concentration of NH_3 catabolic reach a value ($7 \div 10$ mg/L) that have a remarkable influence on the form in which cadmium (II) is present in the system.

Fig. 1 reports two precipitation kinetics of cadmium as carbonate (the average cadmium concentration present in solution versus time) in water

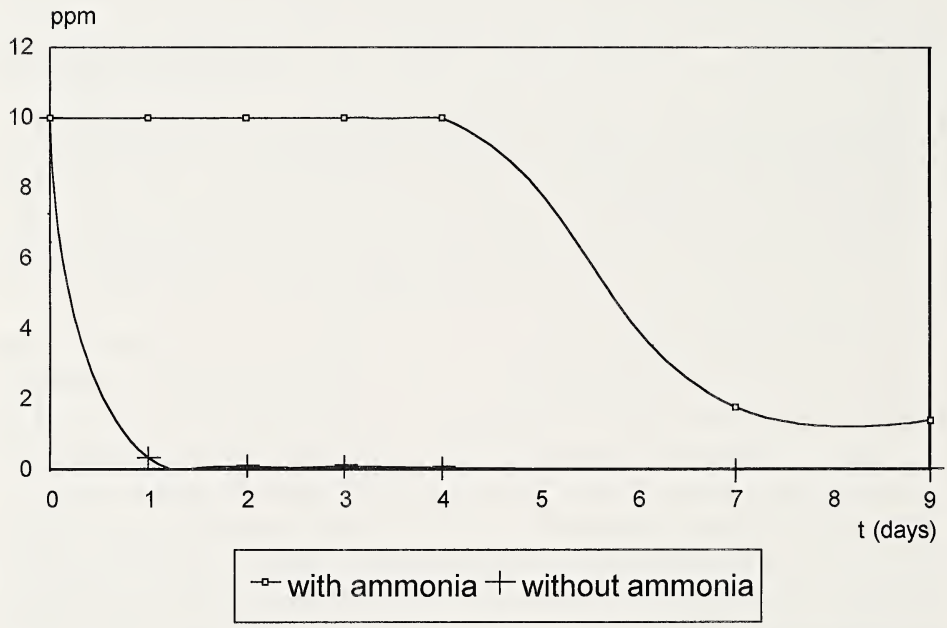

Figure 1 – Storage of ion cadmium in solution with or without catabolic products.

of higher hardness with the *Carassius auratus* and, in comparison, in the same kind of water but without the *Carassius auratus*. It can be noted that the precipitation occurs in significantly different amounts. In point of fact, in hard water and with *Carassius auratus*, we can notice that total cadmium remains in solution longer than four days of the experiment. The precipitation occurs only at the final stage of the experiment, leaving in solution, however, a cadmium concentration of almost 2 ppm. In Kinetic of comparison, always in hard water but without goldfishes, the cadmium carbonate precipitation is immediate with a residual quantity in solution reducing itself to 3.5% of the starting value after 24 hrs. In less hard water,

characterized by greatly lower alkalinity and thus with a lower carbonate cadmium precipitation, no carbonate cadmium precipitation at all occurs, with or without *Carassius auratus*.

The survival, in water of higher hardness, was 100% after 1 hr 30', 50% after 24 hrs, 40% after 72 hrs and 10% after 7 days. Such values are definitely lower, as compared to the results obtained in our previous experiments carried out in waters without ammonia, when survival was almost 100% after the same experiment period of time. (Battaglini *et al.*, data unpublished).

The pathologic anatomy analysis of the dead specimens, has shown haemorrhagic gills, congested liver, haemorrhagic kidneys, and a hyper mucus production all over the body and particularly at gills.

As to the behaviour, fig. 2a reports the average values of beats per min. of the operculum and caudal fin in hard water, and fig. 3a reports the

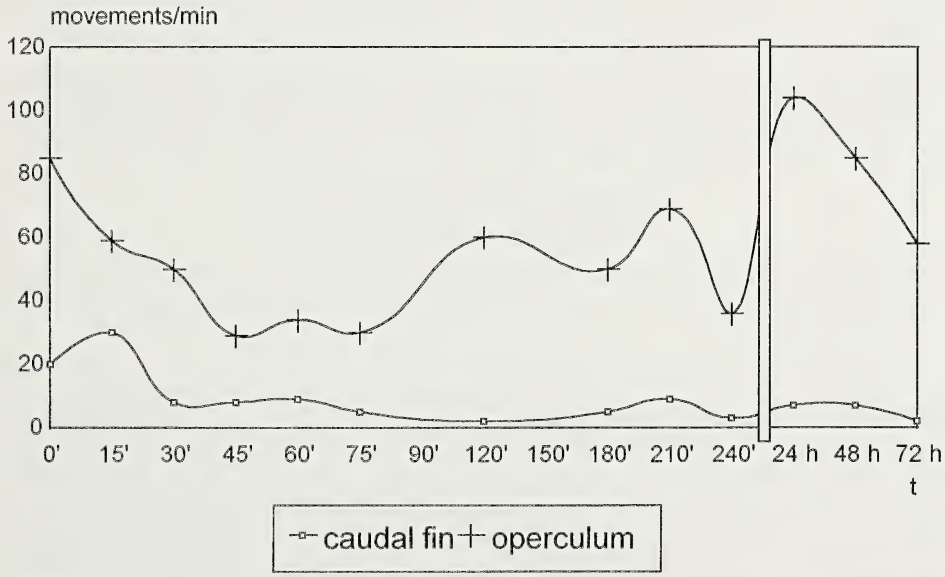

FIGURE 2a – Effect of cadmium + NH_3 on the *Carassius auratus* behaviour in hard water.

relative values in soft water More frequent movements of opercular gill are signs of a greater request of Oxygen (Black 1951; Hughes, 1960). Caudal fin movements are signs of regular activity, decrease or stop are signs of a significant suffering (Bainbridge, 1961). In the controls (Figures. 2b and 3b) the Authors have noted that there is an almost continuous trend having

average values very close to normality (1040 beats per min. of the operculum). As regards the fishes treated (figures 2a and 3a), the values of the operculum beats were noted to be always high but, after a lowering to 60', they turn to be stronger and stronger after 4 hours from the beginning of the experiment.

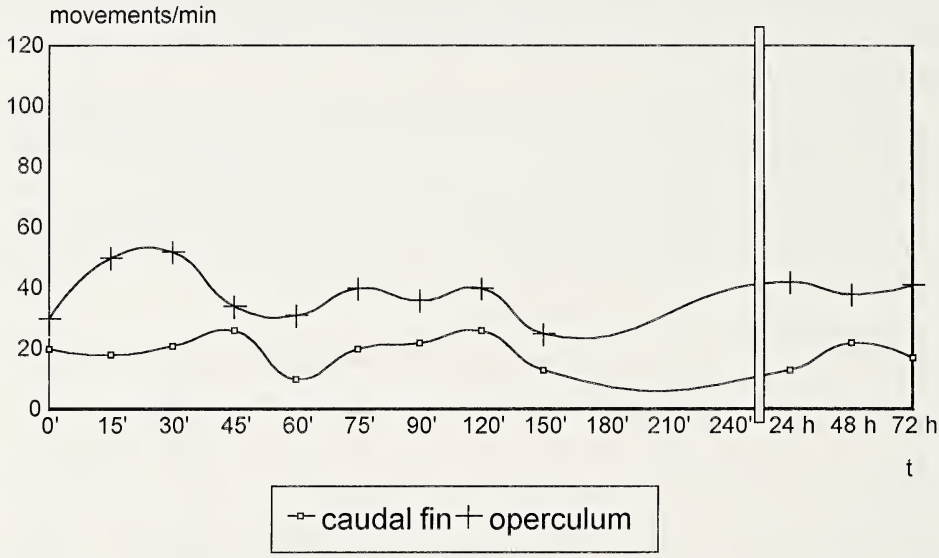

FIGURE 2b – Behaviour of control specimens of *Carassius auratus* in hard water.

The data of cadmium stored up in different organs are characterized by a slight measure error, estimated more or less by 2÷3%, but subjected to a variability of cadmium values depending on the different specimens, and such variability value is much more important. Thus the values reported, which are the average among couples of measures on different samples, are effected by a total uncertainty reaching sometimes a value of even 10%.

Fig. 4a reports cadmium storage values in some organs tested in our experiment, as compared to the same values obtained in a previous experiment (Fig. 4b) (Battaglini *et al.*, 1991), in which no catabolic materials were present when cadmium chloride was added.

The data of atomic absorption confirm that organs having a higher cadmium storage are, as in our previous test: liver, kidney, and gut; while low values or even no value were noted for skin, brain, heart and muscle.

Discussion

In this research, the striking datum for his dramatic and unexpected low value, is the one relative to the survival. Such a dramatic discrepancy could not be expected indeed. In fact, among the different softwater fishes (Flis, 1968) Cyprinids are known to be the least sensitive to NH_3 and

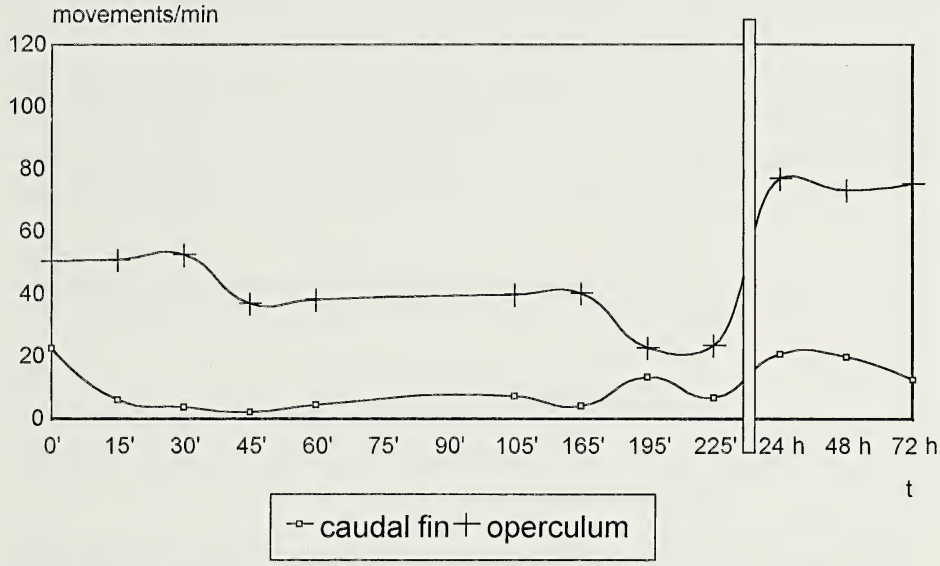

Figure 3a – Effect of cadmium + NH_3 on the *Carassius auratus* behaviour in soft water.

cadmium; on the other hand, the same results obtained in previous experiments suggested definitely different results (Battaglini *et al.*, 1991). This discrepancy is not overcome even if the Authors consider that ammonia itself develops its own toxic action which is to be added to the action of cadmium. In fact, the values of total ammonia measured by the Authors in experiment waters, at the pH values on which we are working, imply the presence of molecular ammonia in hard and soft water of 0.33 and 0.22 mg/L, respectively. These values are definitely lower than the standard value considered toxic for the carp, which is 2.0 mg/L (Malacea, 1968), and for other Cyprinids, like *Pimephales promelas*, which is between 0.75 and 3.4 mg/L of NH_3 (Thurston, Russo & Phillips, 1983).

As a matter of fact, the discrepancy is only apparent and is due to the fact of considering the toxic action of cadmium and ammonia as two

additional phenomena, where, on the contrary, the simultaneous presence of such two substances deeply modifies the chemical nature of both, and consequently, their own toxicity.

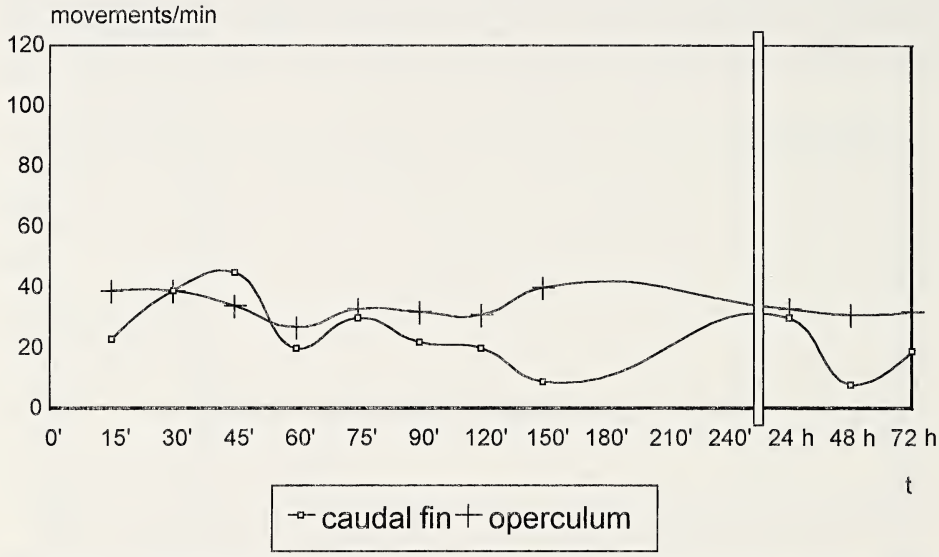

FIGURE 3b – Behaviour of control specimens of *Carassius auratus* in soft water.

To better understand the environment modified by these substances, we must consider the main chemical processes obtained in solution. Ammonia, as already mentioned, is a strong complexing agent of ion cadmium making with this one several compounds up to the tetraminocadmium:

$$4\ NH_{3(aq)} + Cd^{2+}{}_{(aq)} = Cd(NH_3)4^{2+}{}_{(aq)}$$

The progressive involvement of NH_3 in the complex with cadmium, gradually moves the acidbase equilibrium of ammonia towards further molecular ammonia, according to such equilibrium:

$$NH_{4+(aq)} + H_2O = NH_{3(aq)} + H_3O +_{(aq)}$$

and thus, even if at the beginning there is a low amount of molecular NH_3 at the pH level on which we are working, the presence of ion Cd^{2+} is capable of displacing this equilibrium towards the molecular NH_3 just

because of the capture caused by the complexing of the molecules NH_3 produced.

On the other hand, the production of the complexes Cd $(NH_3)^{2+}{}_n$ aims at drastically reducing the concentration of ion Cd2+ in solution, thus highly increasing the solubility of CdCO3 which is produced in calcareous waters with Cd^{2+}:

$$Cd^{2+}{}_{(aq)} + HCO_{3-(aq)} + H_2O = CdCO_{3(s)} + H_3O^+{}_{(aq)}$$

Thus, one of the consequences produced, is the lower and slower precipitation of $CdCO_3$ obtained in experiment tanks with calcareous water and *Carassius auratus*, as compared to the one obtained in water of the same kind but without *Carassius auratus*.

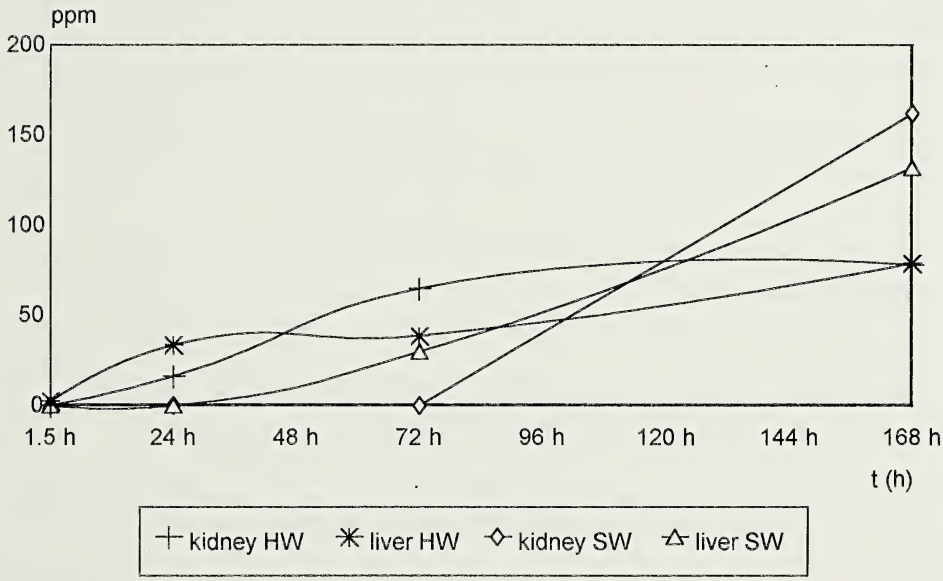

FIGURE 4a – Cadmium storage in liver and kidney of *Carassius auratus* specimens held in hard (HW) and soft (SW) water with ammonia.

Furthermore, we assign to the complexes $Cd(NH_3)_n2+$ a toxicity higher than the one attributed to the single Cd^{2+} and NH_3; in fact, we have ascertained that even in soft water, where cadmium remains in solution for the whole period of the experiment, the survival is, under the same conditions, lower when catabolic ammonia is present together with ion cadmium. In view of the above, also the other data can be better explained.

In this sense, we have shown that the first symptom in goldfish exposed to cadmium + ammonia is respiratory alteration, measured by ventilation frequency, as reported by Maki (1979). Smart (1978) reported hyperexcitability and hyperventilation such as acute toxic mechanism in rainbow trout. Both specimens treated in hard water and those treated in soft water suffer stress which shows an increase of the opercular beats, and a dramatic decrease of the caudal movement, showing the stillness condition of goldfish at the bottom of the tanks. No particular difference can be seen between the two cases, which confirms that both in hard and soft water the *Carassius auratus* during most of the experiment time, are

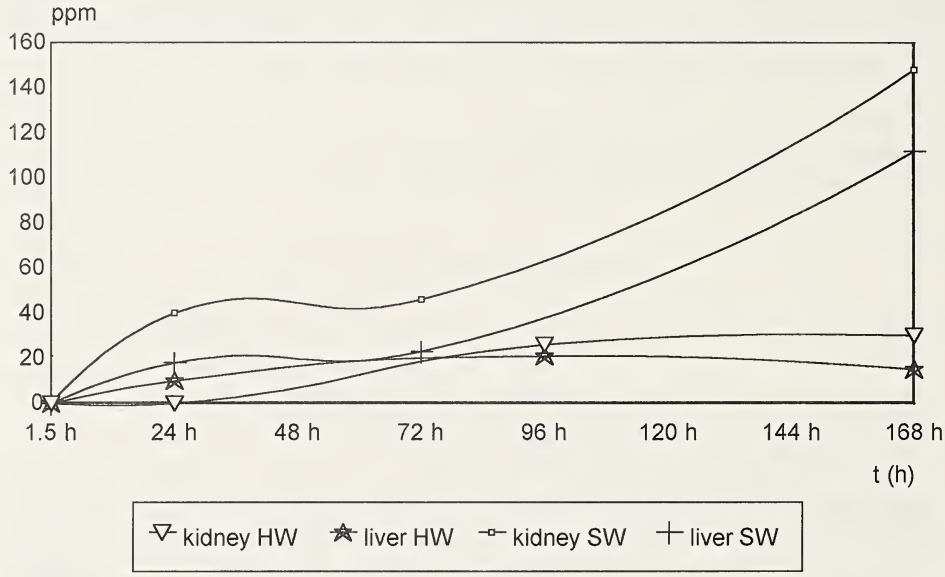

FIGURE 4b – Cadmium storage in liver and kidney of *Carassius auratus* specimens held in hard (HW) and soft (SW) water without ammonia.

exposed to the same amount of cadmium in solution. Similarly, the dead specimens, no matter if placed in hard or in soft water, did shown a mucus hyperproduction which sometimes covered the whole specimen like a shirt. This is in agreement with the relevant literature, since it has been demonstrated that mucus production can be influenced by the presence of harmful environmental factors, such as ammonia pollution (Jakowska, 1963; Dave & Garside, 1976; Zuchelkowski *el al.*, 1981).

As concerns the storage of cadmium data in different organs, the comparison of uptake values obtained in two different water kinds, shows

that, while in harder water the cadmium storage occurs already in the first 24 hrs, in softer water the storage occurs only after a longer time. On the other hand, after 7 days, storage in softer water becomes more important than the one in hard water. For the gut only, higher cadmium values are ascertained in hard waters, even after 7 days, because of the presence in the intestinal lumen of $CdCO_3$ ingested from the bottom of the tank and accumulated there after the fourth experiment day.

Finally, it has to be pointed out that cadmium storages in organs, in the presence of catabolic ammonia, are higher than those obtained without it, both in hard and soft water.

In water of higher hardness, this can be easily explained attributing the higher cadmium storage (in the presence of ammoniacal products) to a longer stay of cadmium solution as a complex which, therefore can be easily absorbed by fishes from solution. Viceversa, the greater storage observed in soft water, brings the Authors to the assumption that cadmium has a higher penetrating capacity into fish tissues when it is coordinated with ammonia, rather than with water molecules.

In conclusion, it can be stated that storage of a catabolic product (such as ammonia) in water will definitely increase cadmium toxicity as regards fish.

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Catabolic NH_3 influence on cadmium toxicity to the gut of *Carassius auratus* L.

Andreozzi G.[1], Antonucci R.[1], Affatato C.[1], De Girolamo P.[1], Gargiulo G.[1], Ferrara L.[2], Sammarco M.[1], Battaglini P.[3]

[1] Dipartimento di Strutture, Funzioni e Tecnologie Biologiche, Università degli Studi «Federico II», via Delpino 1, 80137, Napoli;
[2] Dipartimento di Chimica, Università degli Studi «Federico II», via Mezzocannone 4, 80126 Napoli;
[3] Dipartimento di Zoologia, Università degli Studi «Federico II», via Mezzocannone 8, 80126 Napoli, Italy

Key words: Cadmium, toxicity, gut, immunocytochemistry, *Carassius auratus*.

Abstract. We studied the toxic action that the ion Cd^{2+} exerts on the gut of *Carassius auratus* when the metal is introduced into water containing materials of the fish catabolism including ammonia. The results have shown that in hard water (369 mg/L of $CaCO_3$), the cadmium produces damage to the epithelium of the bulb which appears detached from the connective and an increase of the cellular turnover evidenced by a higher number of the cells in mitosis. On the contrary, in soft water (125 mg/L of $CaCO_3$) the following was noted: a suffering aspect of the epithelium of the bulb, an expantion of the mucous cells and an increase in the mucus mass contained in the intestinal lumen.

Riassunto. Influenza dell'NH_3 catabolica nell'intossicazione da cadmio sull'intestino di Carassius auratus.

È stata studiata la reazione tossica che il cadmio esercita su *Carassius auratus* quando il metallo viene immesso in acqua contenente anche prodotti del catabolismo dei pesci e in particolare tra questi lNH_3. Poiché l'azione tossica del cadmio è correlata alla durezza dell'acqua, sono sta prese in considerazione acque con diversi valori di durezza e precisamente una contenente 369 mg/L di $CaCO_3$ "acqua dura" e un'altra contenete 152 mg/L di $CaCO_3$ "acqua dolce". Sono state allestite due serie di vasche contenenti acqua a diversa durezza. In ciascuna della vasche sono stati immessi 10 carassi. Dopo 8 gg., quando l'acqua si era arricchita dei prodotti catabolici dei pesci, in ogni vasca è stato aggiunto $CdCl_2.2^1/_2H_20$ in quantità pari ad una concentrazione di cadmio nell'acqua di 10 ppm.

L'accumulo di cadmio nell'intestino è stato determinato mediante l'assorbimento atomico previa mineralizzazione dell'organo. Sono state inoltre usate le colorazioni con ematossilinaeosina per l'esame morfologico della mucosa intestinale, e, per la localizzazione e tipizzazione dei mucopolisaccaridi, la reazione PAS, la colorazione con Alcian a pH 2,5 e a pH 1 da sola o associata a digestione con neuroaminidasi. La

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presenza di sostanze Metencefalina like e Leuencefalina like è stata rilevata con metodica PAP.

I risultati ottenuti hanno mostrato che in "acqua dura" con NH_3 la precipitazione del Cadmio inizia solo dopo quattro giorni dall'inizio dell'esperimento. Inoltre, indipendentemente dalla durezza dell'acqua, in presenza di NH_3, l'assorbimento del metallo è sempre elevato, e la sopravvivenza non raggiunge il 15%. Tali dati sono un indice di maggiore tossicità rispetto a nostri precedenti studi su *Carassius auratus* esposti alla stessa quantità di cadmio, ma senza NH_3, ove abbiamo riscontrato valori di assorbimento minori e sopravvivenza del 100%. Le indagini istologiche hanno evidenziato in acqua dura alterazioni della mucosa del bulbo con un aumento del turnover cellulare, numerose mitosi e riduzione delle dimensioni delle cellule mucipare. In acqua dolce, invece, si osservano segni di sofferenza delle cellule epiteliali e dilatazione delle cellule mucipare. Inoltre, sia in "acqua dura" che in "acqua dolce", sono presenti cellule immunoreattive per la Met e la Leuencefalina.

È possibile quindi affermare che, in presenza di NH_3, l'aggiunta di cadmio determina un ambiente molto più tossico. Viene ipotizzato che ciò avviene, non solo perché l'NH_3 si lega al cadmio formando dei complessi che aumentano fortemente la solubilità di quest'ultimo, ma anche perché molto probabilmente le varie specie di cadmio presentano una diversa permeabilità attraverso gli organi bersaglio.

Introduction

In previous researches on the cadmium action concerning the *Carassius auratus* gut kept in strongly calcareous waters the Authors noticed, in shortterm, remarkable histomorphologic changes of the intestinal mucosa and, in longterm, a good recovery capacity of the exposed animals (Andreozzi *et al.*, 1992). Of particular interest was the resulting disappearance, after a treatment of 7 days, of the Met and Leuenkephalinelike immunoreactivity, probably connected to the increase of the mucus production induced by the cadmium (Andreozzi *et al.*, 1992). On the other hand, modifications in the intracellular distribution of a peptide metenkephalinelike have been described in the intestinal epithelium of *Carcinus moenas* contaminated by heavy metals (AmiardTriquet *et al.*, 1986).

Battaglini *et al.* (1991) have reported in *Carassius auratus*, exposed to the cadmium action a remarkable metal uptake in the gut and, furthermore this storage was connected to the different hardness of the experiment water.

On the other hand, the toxic action of the Cadmium varies in relation to the chemicalphysic conditions of the environment (Calamari *et al.*, 1980) and to the presence of other substances that can develop a chelating action towards the ion Cd^{2+} (humic acids, aminoacids, porphyrins, purins) (Ravera, 1984; Sprague, 1987).

Among these substances the Authors focused their attention to ammonia, product of the Carassius auratus catabolism, which stores up in lentic waters in which these fish live. In particular, tke NH_3 is important because it is a strong chelating agent of Cd^{2+} and then it can alter the toxicity both varying the speciation of this metal in the solution and preventing its precipitation as $CdCO_3$ in calcareous waters (Battaglini *et al.*, 1992).

The purpose of the present paper is to study the toxic action of the cadmium in the catabolic NH_3 presence on the *Carassius auratus* gut in waters of different hardness. In particular we will to study the histological and immunohistochemical changes of the intestinal mucosa as well as the changes of some neuropeptides.

Materlals and methods

Animals

The experiment was carried out with waters of different hardness. The first kind, "hard water", was the tap water of Naples (hardness 369 mg/L of $CaCO_3$) and was utilized after an aeration period in a tank for an appropriate dechlorination. The second kind, "soft water", (152 mg/L of $CaCO_3$), was obtained by treating tap water with ionic exchange resins "Soft C" (line "C"), CARMAR, Napoli.

Samples of *Carassius auratus* L., supplied by CARMAR sas (Naples), weighing 6 ± 1 g, have been kept for ten days to get acclimatized, in the different kinds of water in two separate 600 L glass aquaria, equipped with an air pump and a filter (EHEIM, Germany).

To get the experiment 8 tanks (30x30x40 cm) were equipped with an air pump, without filter. During the whole experiment the temperature was constantly at 16 ±1°C, and the photoperiod was 12 hrs.

4 tanks were filled up with 20 L of "hard water" and 4 tanks were filled up with 20 L of "soft water".

4 groups of 10 *Carassius auratus*, randomly chosen in the acclimatization "hard water" glass aquarium, were transferred in the four "hard water" tanks and fed as usual with Tetramin Tetramere, Melle. The same was made for the four "soft water" tanks.

After 8 days in 3 "hard water" tanks and in 3 "softwater" tanks, $CdCl_2.2^1/_2H_20$ was added in such a quantity to obtain a cadmium concentration in water equal to 10ppm in each tank.

In such a way the toxic was added when the experiment water, after 8 days of animal stay, was enriched with catabolic fish materials and among them the NH_3, as we have already mentioned. The specimens from the other two tanks were utilized as control. The cadmium treatment ran out for 7 days

The survival was calculated as percentage of the survived animals seven days after the beginning of the experiment.

Chemical tests

The analyses of the components dissolved in the water were carried out through the traditional methods of volumetric titration (Kolthoff *et al.*, 1973). To determine hardness, calcium and magnesium a standard solution af sodium ethilendiaminotetracetic acid salt was utilized; to get the alkalinity a 0.01N solution of HCl as titrating solution and green bromocresol as acidbase indicator was used; at last for the chlorides a dosage with $AgNO_3$ according to Mohr method was used. All chemical products utilized are Carlo Erba RP reagents, used without any further purification. To determine the pH a pHmeter ORMA, model NK 300 was utilized; to determine the dissolved oxygen was utilized a dissolved oxygenmeter HI 8543 (Hanna Instruments, USA).

The NH_3 produced and present in water was measured with a spectrophotometer using the Nessler reagent to identify the NH_3 (Kolthoffet *al.*, 1973).

The cadmium concentration in solution in the different tanks was regularly measured by an atomic absorption spectrophotometer VARIAN AA275. A parallel precipitation kinetic of Cd^{2+} as $CdCO_3$ was effected in each kind of water used during the experiments, but without fish and relative catabolic materials.

Determination of cadmium uptake

For each group of experimental animals, taken after seven days of exposure, guts ere used to determine the cadmium uptake. Guts were weighed and mineralized by the action of a $HNO_3/HClO_4$ mixture (ratio of concentrated acids 4:1) following K. Bull's method (1975). The resulting solution was brought up to a volume of 10 mL in a calibrated flask and analysed by an atomic absorption spectrophotometer VARIAN AA275 to determine the amount of cadmium.

Histology and Histochemistry

After a treatment of 7 days for each experiment group, 2 *Carassius auratus* were taken and decapitated. At the same time 2 control fishes were sacrificed for each kind of water.

The gut, cranially dissected at intestinal sphincter level and caudally dissected at the rectum end, was completely taken out.

All the specimens, after being fixed in the Bouin's fiuid, were embedded in paraffin and serially cut into 7 μm sections.

To study the mucopolysaccharides the following methods were carried out:

- *Periodic acid Schiff reaction.* PAS was used in order to identify neutral mucopolysaccharides.
- *Alcian blue procedure.* At pH 2.5 (A.B. pH 2.5) and at pH 1.0 (A.B. pH 1.0) the Alcian blue procedure was used alone or in combination with other procedures for the finding and subsequent localisation of acid mucopolysaccharides.
- *Enzyme digestion test.* Sections immediately following the ones stained with A.B. pH 2.5 were digested with neuroaminidase (from *Clostridium perfrigens* type V, Sigma) at a concentration of 0.2% in phosphate buffer for 24 hr at 37°C to put in evidence the sialomucins.

The immunocytochemical tests were carried out by PAP method (Sternberger, 1979) to find out the possible presence of Met and Leuenkephalinelike material.

In both cases the primary antibody was used at 1:500 dilution. Then the reaction product was displayed with 3.3' diamine benzidine (DAB). The reaction specifity was tested both by replacing the primary antibody with PBS and by the absorption procedure.

The control sections were stained with ematoxylineeosine (E.E.).

Results

Survival

The survival analyses showed that after 7 days survival was inferior to 15% both in hard water and in soft water.

Chemical characteristics

Table I reports chemical and physicochemical parameters of two different waters utilized for the experiments. It must be noted that the

presence of catabolic ammonia, at zero time of the treatment, reached a 7 ÷ 10 ppm concentration.

TABLE I – Chemical and physico-chemical parameters of the different waters

Water properties	Hard water	Soft water
Hardness (mg/l $CaCO_3$	369	152
Alcalinity (mg/l HCO_3^-	346	9,8
Ammonia (mg/l NH_3)	7-10	7-10
Chloride (mg/l Cl^-	32	30
Sulphate (mg/l SO_2^{2-})	25	25
pH	7,8	7,6
Dissolved oxygen (mg/l O_2)	> 7	> 7
Temperature (°C)	16 ± 1	16 ± 1

Fig. 1 reports the average concentration of cadmium in solution in the experiment tanks with water having a higher hardness as a function of time

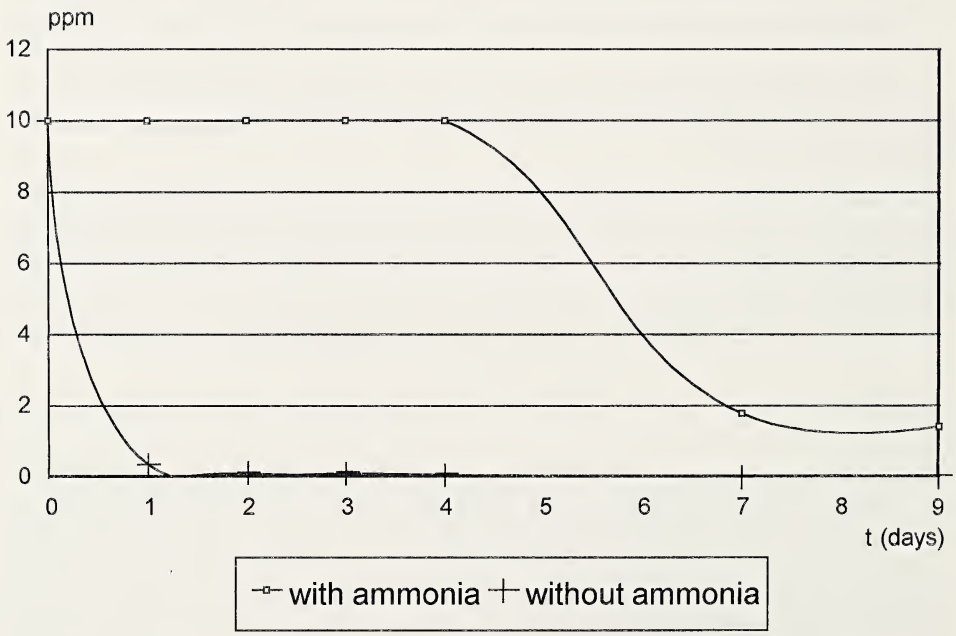


FIGURE 1 – Concentration of C^{d2+} ion in solution in the presence and in the absence of catabolic products.

and, in comparison with the same trend of the cadmium concentration in the same kind of water, but without *Carassius auratus* and the relative catabolic materials. It is easy to note in the two cases that the kinetic of precipitation of $CdCO_3$ is completely different. In fact, in the sample of water for comparison (without *Carassus auratus)* it is possible to note already in the first hours, a fast and practically complete cadmium disappearance from the solution. On the contrary, with the *Carassius auratus* the precipitation occurs only after the fourth day from the beginning of the treatment and, however, in a lower quantity, with a left concentration in solution of almost 2 ppm after 7 days.

Vice versa, in water of lower hardness, the cadmium lasts in solution for the whole length of the test, aside from the *Carassius auratus* presence.

TABLE II – Concentration of Cd^2 in the *Carassius* gut treated for seven days

	with NH_3	without NH_3
Hard water	711	412
Soft water	336	103

Cadmium uptake

Tab. 2 reports the cadmium storage values measured with the atomic absorption spectrophotometry in the gut at the seventh day of the treatment for the *Carassius auratus* treated in two different types of waters. In comparison, the data of a previous research (Battaglini *et al.*, 1992) are reported in the same table, a research relative to the cadmium uptalce in the *Carassius auratus* guts treated for seven days in two different waters having characteristics similar to those utilized in this paper, but at the beginning of the previous research no catabolic ammonia was noted. It can be noticed that the quantity of cadmium in the organ is higher for the *Carassius auratus* treated in water of higher hardness, as observed in the previous experiment. It has also to be underlined that the uptake is always higher when ammonia is present at the beginning, no matter what kind of water is utilized.

Carassius auratus gut structure

The digestive system of the *Carassius auratus* as all Cyprinids is rather simple because it has no stomach.

Thus, in the *Carassius auratus*, the oesophagus is in direct communication with the gut through the oesophageal sphincter. The gut is of uniform size, except for a short cranial part, more dilated, called bulb ofthe gut. (Caceci, 1984).

The gut mucosa, made of the epithelium and the connective tunic, raises in folds having a connective axes (Fig. 2). The epithelium is a single layer of cylindrical cells and contains several mucous cells (McVay and Kaan, 1940) less numerous in the bulb area than the other parts of the gut. They have a typical "drumstick" shape and produce a secretion made of neutral PASpositive mucins and AB pH 2.5 and pH 1.0 positive sulphurated acid mucins, neuroaminidase digestion resistant (Fig.3). Also Metenkephaline (Fig.6) and Leuenkephaline (Fig.9) positive neuroendocrine cells are mixed up with the epithelial cells (Andreozzi *et al.*, 1992). Such roughly pyramidal and open cells are located only in the bulb area and in the most cranial part of the gut.

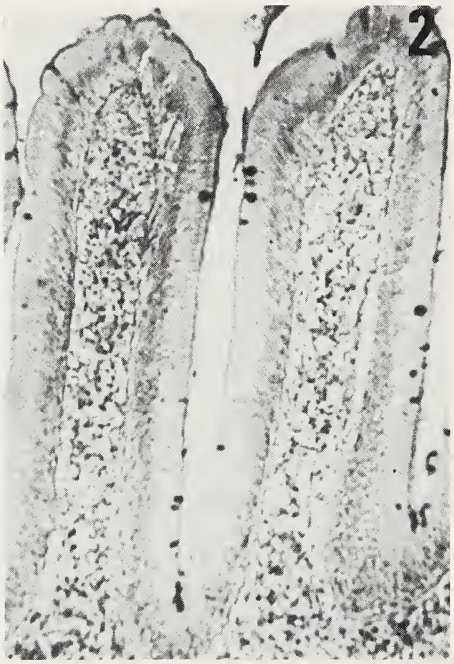


FIGURE 2 – *Carassius auratus* control -intestinal bulb – Alcian pH 2,5240X.

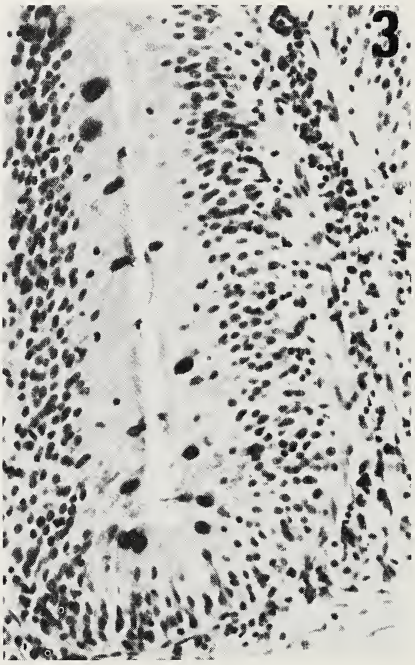
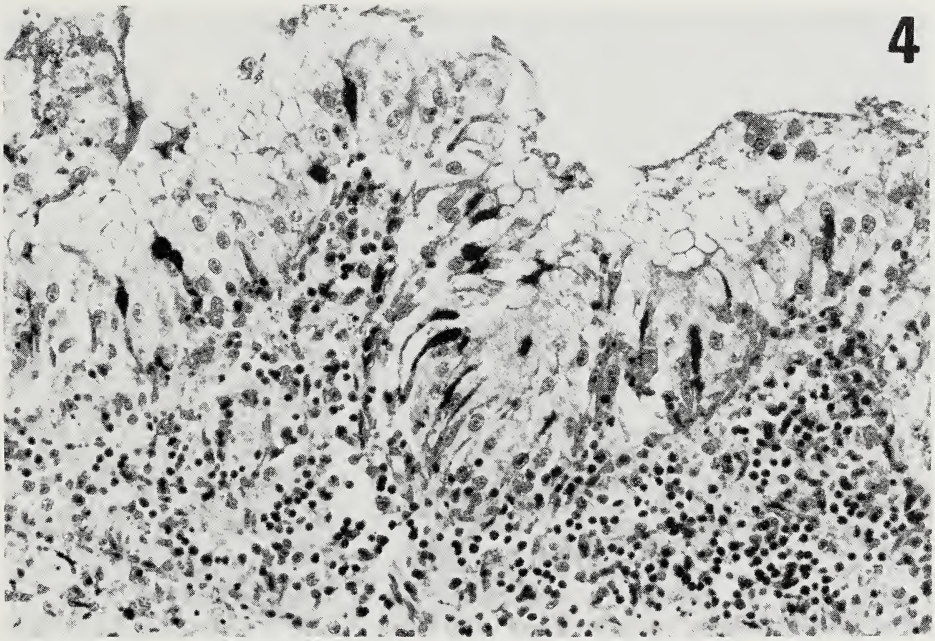


FIGURE 3 – *Carassius auratus* control -intestinal bulb – Alcian pH 2,5380X.



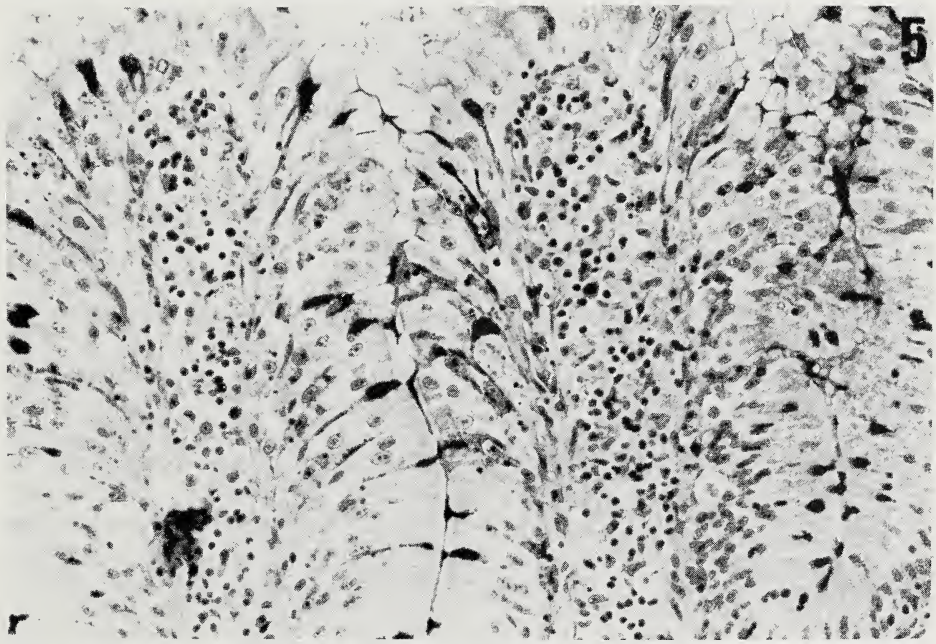


FIGURE 4 and 5 – *Carassius auratus* treated with Cd^{2+} in hard water for seven days – intestinal bulb – Alcian pH-3,5. Note very thin mucous cells and cells flanking away in the top of the villus – 600X.

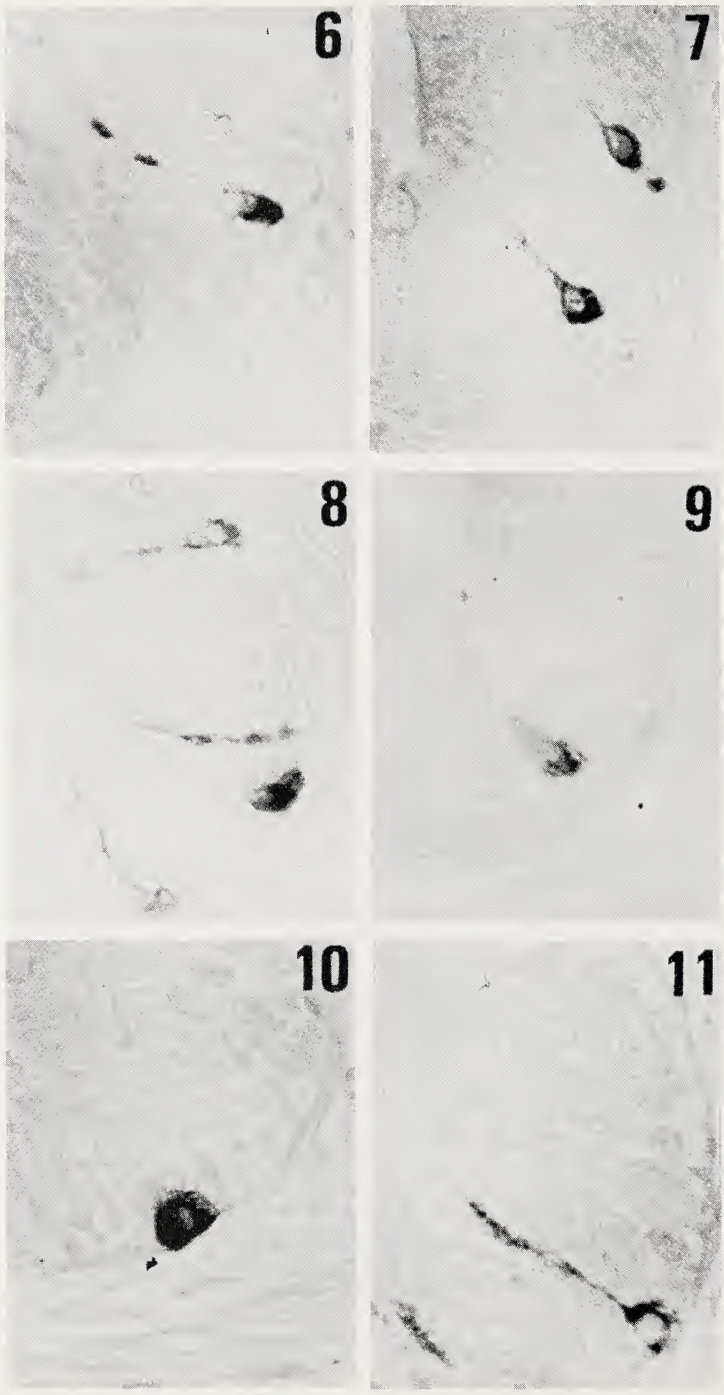


FIGURE 6 – *Carassius auratus* control – intestinal bulb – metenkephalin 800X

FIGURES 7 and 8 – *Carassius auratus* treated with Cd^{2+} in hard water for seven days – intestinal bulb – metenkephalin 800X

FIGURE 9 – *Carassius auratus* control – intestinal bulb – leuenkephalin 800X

FIGURES 10 and 11 – *Carassius auratus* treated with Cd^{2+} in hard water for seven days – intestinal bulb – leu-enkephalin 800X

Hard water experiment

In the animals exposed for seven days to the cadmium action in hard water at the presence of catabolic ammonia, the gut mucosa appears to be rather damaged at the bulb level and at the more cranial part of the gut. In fact, in these areas the epithelium is often detached from the inferior connective and it was noticed that some cells were flaking away. (Fig. 4, 5). The mucosa keeps a normal aspect only at the bottom of the folds and at the remaining part of the gut. The mucous cells, often much more elongated and thinned as compared to those of the controls, have positive granules scattered in the whole cytoplasm up to the base. In addition there are several mitosis.

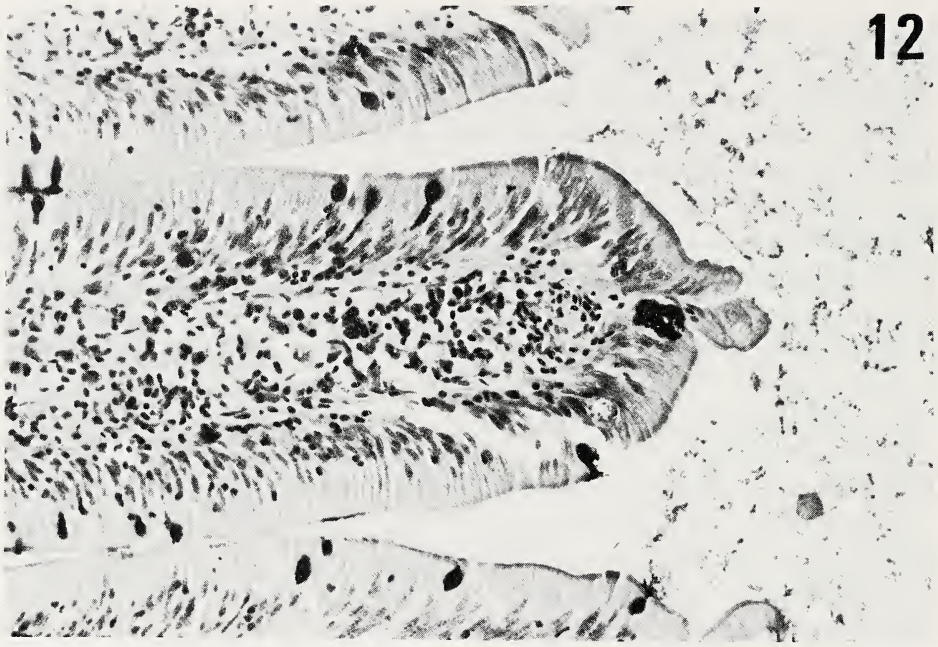
The histochemical reactions for the mucopolysaccharides have shown, as for the controls, the presence of positive A.B. pH 2.5 and pH 1.0 positive acid mucins and neuroaminidase mucins resistant. There are also cells containing PAS positive neutral muans.

The immuno reactive Metenkephaline cells seem to be more numerous and more significantly positive as compared to the controls (Figures 7, 8). Their shape and distribution result unchanged. The IR (immunoreactive) cells for the Leuenkephaline seem to be more significantly positive as compared to the controls (Figures 10, 11). Their number and shape are similar to those of the controls.

Soft water experiment

In the gut of the animals treated for seven days with cadmium in softwater with catabolic ammonia, the bulb mucosa appears suffering. In fact, the epithelial cells appear coarted and the mucous cells located at the top of the folds are often so swollen as to look like a "ball" or a "bag" with a highly thickened secretion. Sometimes it seems that the nearby cells merge themselves (Figures. 12, 13). Furthermore there is an increase of the mucus mass contained in the intestinal lumen. The histochemical reactions have shown, as in the controls, the presence of PASpositive mucins and A.B.positive mucins, neuroaminidase resistant.

Positive Metenkephaline and Leuenkephaline cells were found and their frequency and distribution were similar to those found in the animals of controls.



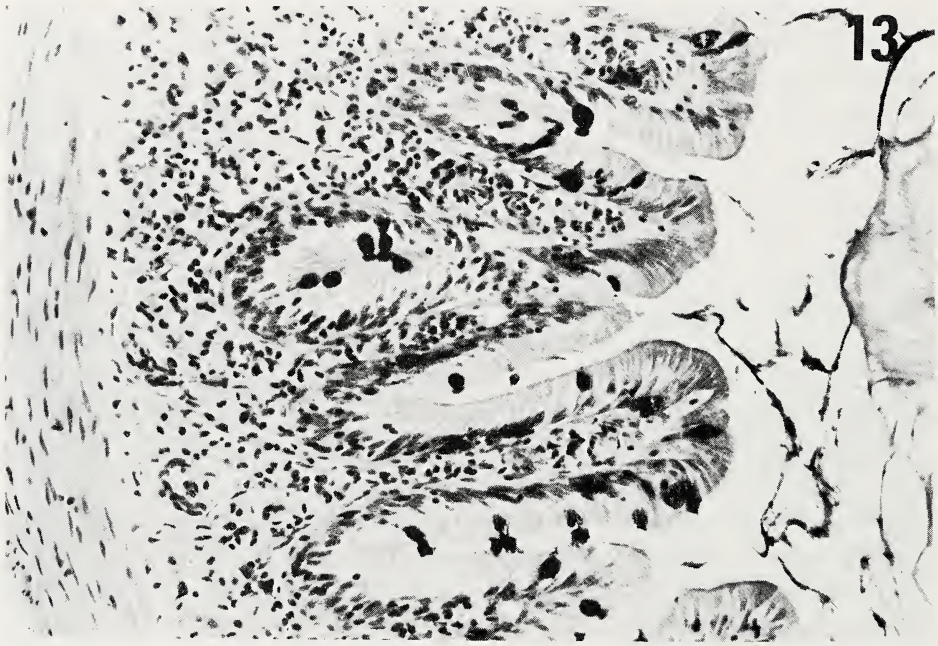


FIGURE 12 and 13 – *Carassius auratus* treated with Cd^{2+} in soft water for seven days – intestinal bulb – Alcian pH 2,5 – Note the mucous cells enlarged and sometimes merged (arrows). 380X.

Discussion

The results obtained on the *Carassius auratus* after 7 day treatment, suggest that the cadmium shows a higher toxicity in the presence of catabolic NH_3. In addition, unlike what was noticed in our previous researches (Battaglini *et al.*, 1991) this action is not strictly influenced by the water hardness.

The most significant results obtained in this experiment are those concerning the survival. In fact, in the present experiment, after 7 days the survival had not reached the value of 15%. On the contrary, in our previous experiments on *Carassius auratus*, we have reported that with equal cadmium concentration and lack of catabolic NH_3, a survival up to 100% after 40 days in hard water (520 mg/L of $CaCO_3$) (Battaglini *et al.*, 1992) and up to 90% after 40 days in soft water (150 mg/L of $CaCO_3$) (not yet published data).

The histological researches, after 7 days of exposure to cadmium with catabolic ammonia, have focused significant changes of the intestinal mucosa, in particular at the bulb level, with changes of the mucus storage and mucous cell shape. In the specimens treated in "hard water" the mucous cells appear thinner and elongated, with positive granules up to the basal area, while in the specimens treated in soft water they are very swollen "bag" shaped with a very thickened secretion. In both cases the immunoreactive Met and Leuenkephaline cells are positive and more numerous in the specimens treated in hard water.

These data differ from those described in our previous researches (Andreozzi *et al*, 1992; Andreozzi *et al.* 1991a, 1991b). In highly calcareous waters the AA have observed, after a treatment of 7 days with cadmium, less damage at the gut mucosa level and the disappearance of immunoreactivity to Met and Leuenkephaline; in a longer period a remarlcable capacity of restoring the histological and immunocytochemical features was noted.

Thus, a higher toxicity of cadmium in the presence of catabolic NH_3 is evident and it appears with different characteristics in environments having different hardness. In fact, while in hard water the numerous mitoses, the detached epithelium and the reduced sizes of the mucous cells induce us to think of a turnover cell increment, on the contrary, in soft water the coarted aspect of the epithelial cells is a sign of a suffering tissue condition, while the increase of the mucous cell size and a greater mucus quantity in the intestinal lumen make us suppose a defensive reaction of the epithelium itself.

These data match with those described in *Fundulus* by Gardner and Yevich (1970) and in *Salmo gairdneri* by Crespo *et al.* (1986) influenced by cadmium alone. In fact, these authors describe the cellular "suffering" and the mucus secretion increase. In addition, Crespo *et al.* (1986), always in trout, describe also an increase in the mitoses number. The presence of immunoreactive cells for the Leu and Metenkephaline, after 7 day treatment, in contrast to what reported by Andreozzi *et al.* (1992) could be related to an emergency situation from which the animal cannot recover any longer.

On the contrary, the atomic absorption data in the gut after 7 days of treatment show a higher metal storage in the specimens treated in hard water compared with those treated in soft water. This datum is similar to what previously reported in goldfish by Battaglini *et al.* (1991) on the action of the cadmium in waters of different hardness, without catabolic ammonia.

In this case too, the higher value in hard water is likely to be attribute to the ingestion, even if in a lower quantity, of $CdCO_3$ which begins to precipitate on the bottom after four days of experiment. In soft water, at the same cadmium quantity dissolved in solution, the presence of NH_3 determines a clear increase of metal storage in the gut as compared to what reported in specimens exposed only to the cadmium (Battaglini *et al.*, 1991).

We can assert now that to the same quantity of cadmium added the "experimental environment" created results to be definitely more toxic. The reasons of such a higher toxicity can be, in our opinion, attributed just to the presence of NH_3 expelled by the fishes as catabolic material. In fact, the first effect of the molecular ammonia is to bind itself to Cd^{2+} producing several complexes that strongly increase the $CdCO_3$ solubility developed in calcareous waters with Cd^{2+}.

In fact, in test tanks with the goldfish and their catabolic materials, the $CdCO_3$ precipitation is slower and inferior as compared to that which occurs in waters of the same kind but without *Carassius auratus*. Thus, the presence of catabolic ammonia determines a cadmium permanence in hard water as we observed in soft water.

On this matter, a first hypothesis would lead us to state that, in waters of higher hardness, the highest toxicity of Cd^{2+} with NH_3 can be connected to the higher and longer presence of the toxic in solution than in the case in which ammoniacal materials. are lacking. But this element, even if important, cannot explain why even in water of less hardness where the $CdCO_3$ precipitation does not occur it is possible to obtain a definitely

lower survival with respect to that obtained in absence of NH_3. As in this second case, the *Carassius auratus*, with or without NH_3, are in contact with the same total amount of cadmium dissolved in solution (10 ppm) and are in environmental conditions almost similar, the different survival observed could be ascribed to the different cadmium speciation in solution and very likely to a different permeability, of different forms in which cadmium is present in solution, through the organ targets of the test animals.

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CONTRIBUTI IN ITALIANO

Mammiferi olocenici provenienti da uno scavo effettuato nell'isola di Capri (Italia meridionale)

Barbera C., Leuci G., Rapuano M., Virgili A.
Dipartimento di Scienze della Terra,
Università degli Studi «Federico II», L.go San Marcellino 10, 80138 Napoli, Italy

Riassunto. In questo lavoro si segnala la presenza di una mammofauna olocenica rinvenuta durante uno scavo recente nell'isola. Associati a reperti ceramici di età romana sono stati rinvenuti resti di *Equus, Sus, Capra, Ovis, Bos* e gallinacei. Per la prima volta, viene segnalata la presenza del *Bos primigenius*, specie non facente parte delle faune endemiche dell'isola.

Gran parte dei reperti mostra chiari segni di bruciatura che sono riferibili ad avanzi di pasto, appartengono sia ad animali giovani che ad animali adulti ad eccezione di quello riferito al cavallo che sicuramente è appartenuto ad un individuo adulto.

Premessa

Questa nota si pone come obbiettivo l'identificazione di alcuni resti ossei rinvenuti nell'isola di Capri, in uno scavo effettuato a scopo edilizio in via Truglio 19.

Lo studio di questi reperti ha portato ad identificare la specie *Bos primigenius* Boj, mai segnalata prima nell'isola.

I resti dei mammiferi in oggetto sono depositati presso il Centro documentale A. Ciccogliano di Capri e siglati con la lettera C seguita da numeri progressivi.

Precedenti conoscenze

Sulla zona esistono pochi dati bibliografici sia dal punto di vista geologico che paleontologico specialmente per i sedimenti della copertura pleistocenica.

Received 14.5.92, accepted 27.11.92.

Rellini (1910) riconosce, nel giacimento Quisisana-Certosa ritrovato casualmente durante uno scavo per fondazioni edilizie e segnalatogli da E. Cerio, appassionato collezionista e raccoglitore di tutto il materiale racchiuso nel museo di Capri che porta il suo nome, una parte superiore di età olocenica con presenza di ceramiche del bronzo ed industrie del Neolitico medio, una intermedia di età pleistocenica-terminale presentante intercalazioni di terra rossa con resti di cervidi e di una parte inferiore (Pleistocene medio) con industrie litiche acheuleane e resti di grandi mammiferi.

Bassani e Galderi (1911) verificano la stratigrafia dello scavo di Quisisana creando una apposita sezione ad esso adiacente.

Azzaroli (1961) nel suo lavoro sul nanismo dei cervidi insulari dopo aver rivisto il materiale del livello intermedio di Cerio, Pigorini e Rellini (1906), descrive una nuova specie di cervide nano dell'isola di Capri come *Cervus tyrrenicus*.

Successivamente Piperno e Segre (1984) propongono una stratigrafia più aggiornata dei giacimenti Quisisana-Certosa e identificano cinque livelli, che dall'alto in basso sono:

1) Suoli e detriti non consolidati.
2) Pozzolane stratificate del Pleistocene superiore finale.
3) Terra rossa.
4) Tufo grigio campano, facies cineritica.
5) Argilla rossa con industria acheuleana e grandi mammiferi.

Cinque, Gliozzi ed Esu (1988) e successivamente Gliozzi (1989) riconoscono in un riempimento della grotta "Vascio o' funno" localizzata sul lato SudEst dell'isola, una nuova specie di roditore *Apodemus silvaticus thyrrenicus* con un cranio di grandi dimensioni e denti arcaici.

Barbera e Cimmino (1990) in materiale subattuale di una grotta del versante orientale di Monte Solaro riconoscono resti di *Suncus etruscus* (SAVI), Rattus rattus *(L.)*, Mus domesticus RUTTY, *Apodemus sp.*, *Eliomys quercinus* (L.). Alcune di queste specie, *Suncus etruscus* ed *Eliomys quercinus* non erano mai state segnalate nell'isola.

Serie stratigrafica

Lo scavo in via Truglio, come da premessa, è stato effettuato quale sbancamento per uso edilizio. Esso ha raggiunto una profondità di circa 3,50 m e la successione dei sedimenti affioranti dall'alto verso il basso è la seguente:

m. 2,80 – terreno di copertura agraria
m. 0,50 – ceneri vulcaniche
m. 0,80 – pozzolane stratificate
m. 0,30 – intercalazioni di terra rossa

Data la vicinanza del sito di provenienza del materiale allo scavo di Quisisana-Certosa descritto da Piperno e Segre (1984) è stato possibile confrontare le colonne stratigrafiche relative ai due giacimenti si è così notato che il materiale proveniente da via Truglio si rinviene in un livello che corrisponde al livello 3 (intercalazione di terra rossa) del giacimento di Quisisana.

Probabilmente se lo scavo non fosse stato interrotto sarebbe stato possibile raggiungere il livello 5 con i grandi mammiferi e l'industria litica di età acheuleana.

Descrizione del materiale

Per la descrizione del materiale viene usata la classificazione di Grzimek (1974). Tutto il materiale è stato confrontato con quello presente nei Musei Universitari Napoletani.

Essendo la quasi totalità dei reperti in frammenti, i parametri delle tabelle sono riferiti alle ossa sulle quali è stato possibile effettuare misurazioni.

Caballus caballus (L.)

Materiale esaminato:

Un solo frammento di tibia di grandi dimensione appartenente ad un individuo adulto (C1).

Numero di esemplari 1.

Sus scrofa (L.)

Materiale esaminato.

2 canini sinistri, 1 superiore (C2) ed 1 inferiore (C3)
1 canino destro superiore (C4)
1 incisivo mandibolare sinistro (C5)

2 PM superiore sinistri (C6, C7)
1 M2 superiore sinistro (C8)
2 frammenti mascellari, 1 sinistro (C9) ed 1 destro (C10)
4 frammenti mandibolari, 3 sinistri (C11, C12, C13) ed uno destro (C14)
2 omeri destri frammentari (C15, C16)
1 omero sinistro frammentario (C17)
1 femore destro frammentario (C18)

Numero minimo di individui: tre.

Osservazioni:

Due delle mandibole esaminate appartengono ad un individuo di età avanzata (C 11, C 14), mentre una delle mascelle appartiene ad un individuo di giovane età. Anche i canini appartengono ad individui giovani. Le ossa lunghe sono rotte a livello della diafisi.

I parametri sono riportati in tab. I e II.

TAB. I – Parametri morfometrici dei denti: *Sus scrofa* LINNEO

masc. dx c10	h	diam. meso-dist.	diam. vest.-lingu.
PM4	0,59	1,09	1
M1	0,33	1,38	1,31
M2	0,67	1,85	1,17
masc. dx c9	h	diam. meso-dist.	diam. vest.-lingu.
PM4	0,83	1,17	0,86
M1	0,66	1,5	1,13
M2	0,99	1,7	1,24
masc. dx c11	h	diam. meso-dist.	diam. vest.-lingu.
PM4	0,27	1,59	1,05
M1	0,76	1,54	1,85
M2	0,35	1,82	1,18
denti isolati	h	diam. meso-dist.	diam. vest.-lingu.
I C5		0,53	0,3
P M2 inf. dx C7	1,15	1,23	0,77
P M2 inf. sx C6	1,26	1,28	0,45
M 2 sup. sx C8	0,9	1,23	1,12

TAB. II – Parametri morfometrici delle ossa: *Sus scrofa* LINNEO

OMERO: misure longitudinali	C 15	C 16
Diametro trasversale max estremità inf.	3,93	3,25
Diametro trasversale inf. troclea (f. post.)	2,77	2,58
Diametro trasversale sup. troclea (f. anter.)	1,5	1,2
Diametro verticale max troclea	3,62	3,12
Diametro trasversale sup. artic. (f. anter.)	2,82	2,29
Lunghezza foro oleocraneo	0,67	0,56
Larghezza foro oleocraneo	0,57	0,54
FEMORE: misure trasversali	C 18	
Lunghezza collo	1,78	
Diametro trasversale della testa	2,47	
TIBIA: misure trasversali	C 17	
Diametro trasversale epifisi inf.	2,88	
Diametro antero-post. sup. art. inf.	1,74	
Diametro trasversale sup. art. sup.	1,9	

Capra hircus (L.)

Materiale esaminato.

1 incisivo destro (C19)
2 M1 inferiori destri (C20, C21)
2 M2 inferiori destri (C22, C23)
1 frammento di atlante (C24)
3 frammenti mandibolari, due destri (C25, C26) ed uno sinistro (C27)
1 frammento di radio destro (C28)
2 frammenti di coxale sinistro (C29, C30)
1 frammento di tibia destra (C31)

Numero minimo di esemplari: due.

Osservazioni:

Tutte le mandibole, per quanto frammentarie presentano la serie dentale completa dai premolari.

Alcune mandibole appartengono ad individui giovani (C26, C27), in quanto le cuspidi sono poco usurate.

Le ossa lunghe sono rotte all'altezza della diafisi.

I parametri sono in tabella III e IV.

TAB. III – Parametri morfometri dei denti: *Capra hircus* LINNEO

mand. dx C25	h	diam. meso-dist.	diam. vest.-lingu.
PM1	0,94	0,54	0,53
PM2	1,02	0,74	0,67
PM3	1,11	1	0,78
M1	1,13	1,25	0,83
M2	1,18	1,61	0,93
M3	1,27	2,37	0,91
mand. sx C26	**h**	**diam. meso-dist.**	**diam. vest.-lingu.**
PM2	1,07	0,82	0,58
PM3	1,16	0,93	0,6
M1	1,55	1,33	0,77
M2	1,46	1,51	0,84
M3	1,36	1,7	0,77
mand. sx C11	**h**	**diam. meso-dist.**	**diam. vest.-lingu.**
PM2	1	0,71	0,61
PM3	1,1	0,84	0,62
M1	1,08	1,05	0,72
M2	1,31	1,31	0,87
M3	1,58	2,25	0,93

Ovis sp. vel *Capra* sp.

Materiale esaminato:
1 calcagno sinistro (C32).
Numero minimo di esemplari: uno.

Osservazioni:
Alcune rugosità decorrono dalla destra del corpo dell'osso sino alla suspentaculum, ciò fa pensare che esso dovesse appartenere ad un animale selvatico.

TAB. IV – Parametri morfometrici delle ossa: *Capra hircus* LINNEO

RADIO: misure trasversali	C 28	
Diametro trasversale della testa	2,57	
Diametro trasversale del collo	2,05	
Diametro trasversale fossetta radiale	1,83	
Diametro antero-posteriore della testa	1,56	
RADIO: misure longitudinali	C28	
Diametro antero-post. oleocraneo	1,05	
Diametro antero-post. del corpo	0,52	
Diametro antero-post. apofisi stiloide	0,52	
BACINO: misure trasversali	C 29	C 30
Diametro branca discendente pube	2,41	2,74
Diametro antero-post. cavità cotiloidea	2,54	
TIBIA: misure trasversali	C 31	
Diametro trasversale del corpo	1,43	
Diametro trasversale epifisi inf.	2,88	
Diametro antero-posteriore sup. art. inf.	1,32	
Diametro trasversale sup. art. sup.	1,82	

Bos primigenius BOJ

Materiale esaminato:

1 M1 superiore destro (C33)
1 porzione prossimale di ulna destra (C34)
1 metatarso destro rotto longitudinalmente e trasversalmente (C35)
1 II falange interna destra (C36)

Numero minimo di individui: almeno due in quanto la porzione prossimale di ulna appartiene ad un individuo di grandi dimensioni, mentre il metatarso appartiene ad un individuo adulto le cui dimensioni rientrano tra le minori della specie.

Osservazioni:

Sono stati fatti diversi tentativi di ricercare dei caratteri diagnostici nei denti isolati per distinguere il bove dal bisonte. Boule, 1906, indica come

caratteristica la forma più quadrata dei molari del bisonte. Altri AA. insistono di più sui caratteri del solo M3 della mandibola (Boessneck *et al.*, 1963). Tuttavia i molari del bisonte sono più quadrati di quelli del bove; il lato labiale è meno appuntito, la copertura di smalto più forte. Il dente del bisonte effettivamente, è reso più corto e tozzo dalla diversa morfologia delle pieghe laterali e da una differente forma e posizione del talonide (Corridi, 1987). I confronti effettuati con materiale presente nel Museo di Paleontologia dell'Università di Roma hanno permesso di attribuire ad un M1 superiore destro di *Bos primigenius* BOJ il reperto (C33) da noi esaminato.

La porzione prossimale dell'ulna destra (C34) appartiene ad un individuo giovane, dato il tipo di ossificazione, ma di grandi dimensioni; manca della tuberosità superiore e della porzione prossimale del becco dell'olecrano. Il reperto C35 è la porzione mediale di metatarso destro che oltre a rotture dovute all'estrazione probabile del midollo, presenta caratteristiche striature dovute all'azione dell'uomo.

I parametri sono in tabella V.

TAB. V – Parametri morfometrici delle ossa: *Bos primigenius* BOJ

SECONDA FALANGE int. dx: mis. longitud.	C 36
Lunghezza massima	4
Altezza massima	2,08
SECONDA FALANGE int. dx: mis. trasvers.	**C 36**
Diametro trasversale sup.	2,88
Diametro antero-posteriore estr. sup.	2,9
Diametro trasversale troclea	1,93
Diametro trasversale estr. inf.	2,24
Diametro antero-posteriore condilo interno	2,1
Diametro antero-posteriore condilo esterno	2,85
Diametro trasversale	2,44
Diametro antero-posteriore	2,23

Aves sp. et gen. ind.

Tra il materiale da noi esaminato sono stati rinvenuti frammenti lunghi di ossa di gallinacei. Particolarmente ben conservati appaiono:

1 cubito destro (C37)

1 tibia sinistra (C38)

1 metatarso sinistro (C39)

Numero minimo di esemplari: uno.

Osservazioni:

I gallinacei osservati sono di discrete dimensioni. Il cubito ed il metatarso sono in buone condizioni di fossilizzazione, la tibia si presenta rotta a circa i due terzi del corpo.

I parametri sono in tabella VI.

TAB. VI – Parametri morfometrici delle ossa: Gallineacei

TIBIA: misure longitudinali	C 38
Lunghezza della cresta	2,47
TIBIA: misure trasversali	**C 38**
Lunghezza incavo popliteo	0,7
Diametro trasversale del corpo	0,92
Diametro trasversale max estr. inf.	1,6
METATARSO: misure trasversali	**C 39**
Diametro trasversale estremità sup.	1,45
Diametro antero-posteriore estr. sup.	1,05
Diametro trasversale del corpo	0,78
Diametro trasversale epifisi inf.	1,34
Diametro antero-posteriore epifisi inf.	0,37

CONCLUSIONI

L'insieme faunistico, seppure con i limiti dovuti alla tipologia del giacimento ed al metodo alquanto sommario di recupero può dare alcune informazioni sulla popolazione mammaliana dell'isola di Capri nel tardo Olocene.

I grandi mammiferi come: l'uro, *Bos primigenius* BOJ, erano ivi rappresentati e probabilmente anche utilizzati dall'uomo sia come cibo che per il lavoro nei campi. Come cibo probabilmente venivano utilizzati gli altri animali costituenti la fauna (cinghiale, pecora. capra ecc.).

Gran parte dei resti mostrano tracce di bruciature e tagli longitudinali, caratteristici dell'opera dell'uomo, tesi all'estrazione delle parti edibili in essi contenuti.

Gli scarsi resti di ceramica, associati alle ossa che abbiamo fatto esaminare da colleghi archeologi della sovrintendenza ai Beni AA. di Napoli e Caserta ci hanno dato un arco temporale compreso tra il I° secolo

A.C.: ed il II° D.C., periodo questo della urbanizzazione da parte dei romani dell'isola di Capri. L'interesse di questa nota resta quindi principalmente limitato alla prima segnalazione certa, con resti accertati di *Bos primigenius* BOJANUS in epoca romana nell'isola di Capri.

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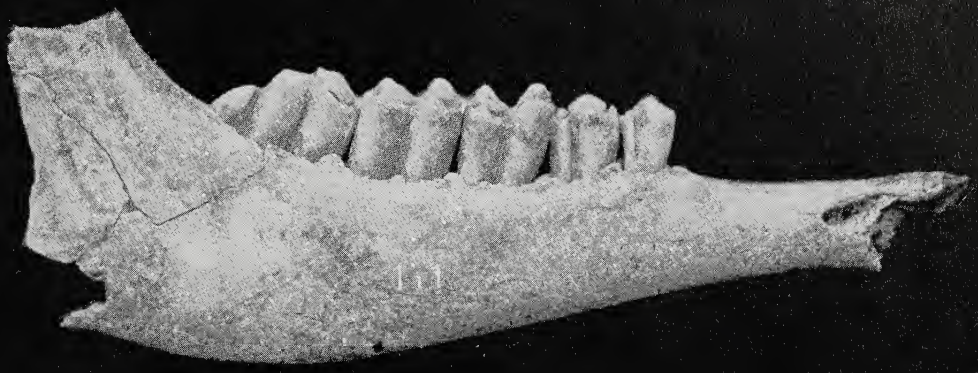
TAVOLE

TAV. 1 – Capra hircus LINNEO.
1. Emimandibola sinistra (C 26). Norma labiale.
2. Emimandibola sinistra (C 27). Norma linguale.
3. Emimandibola destra (C 25). Norma labiale.

TAV. 1

1

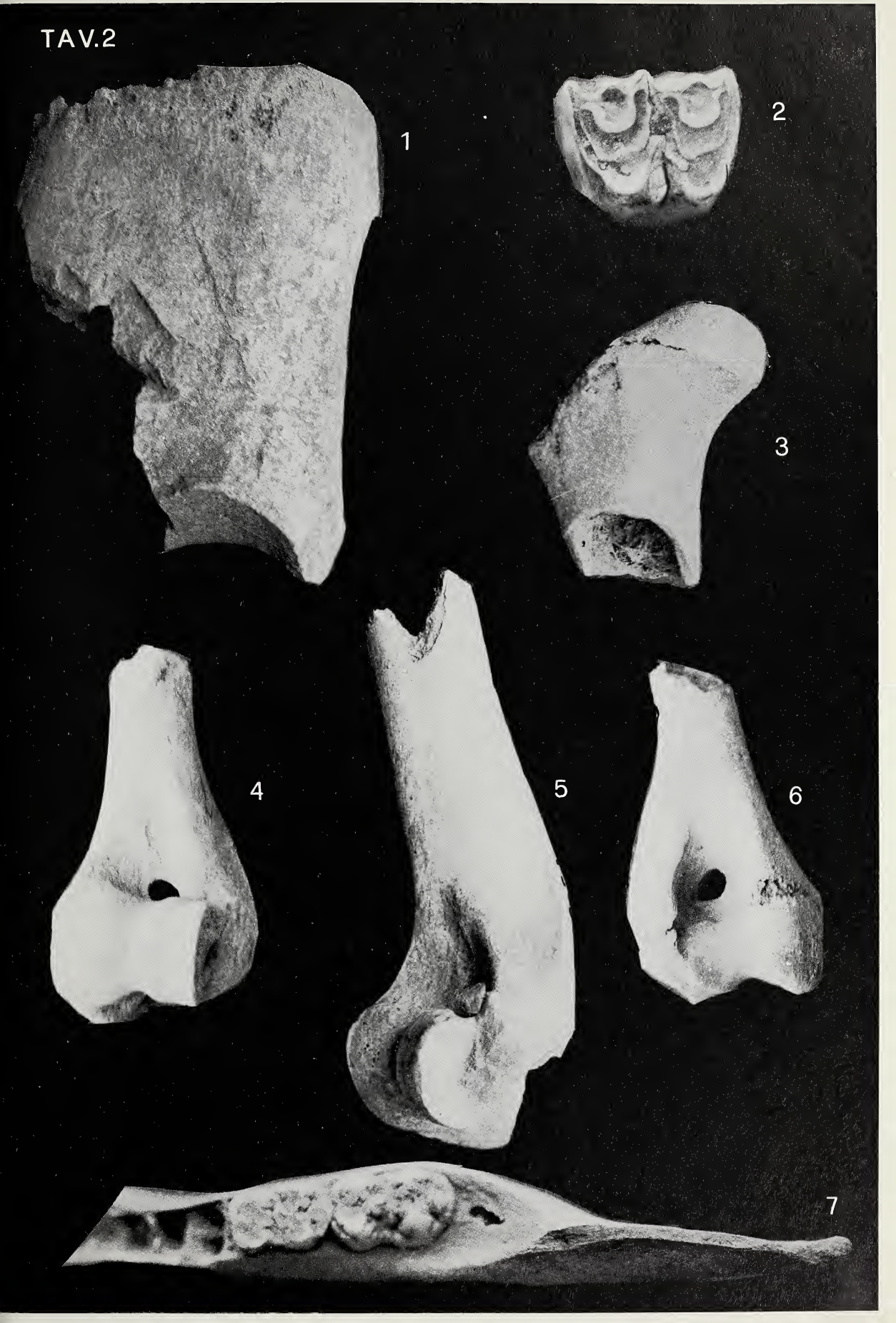


2

3

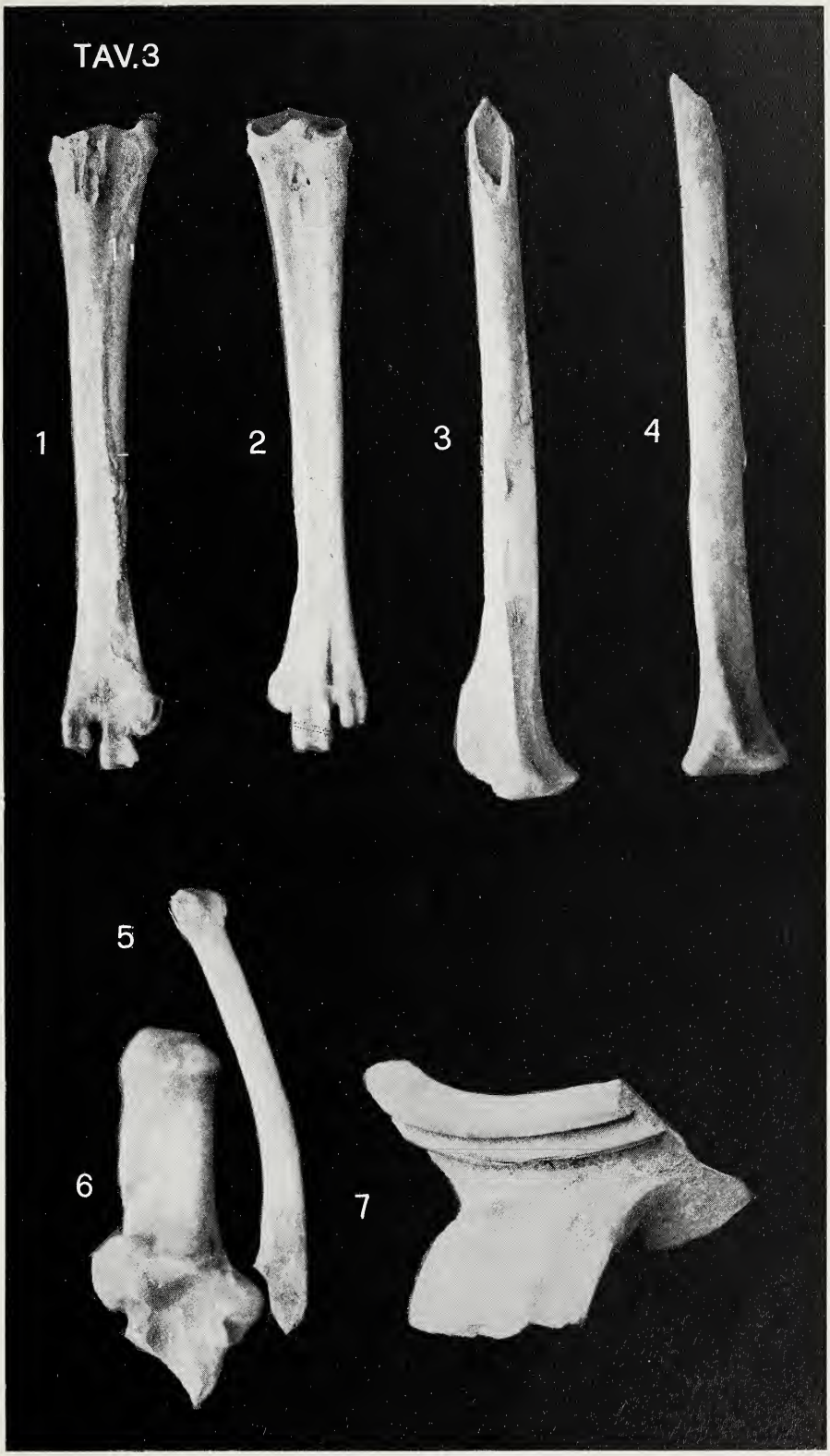
TAV. 2 – a) *Bos primigenius* BOJ.
1. Porzione prossimale di ulna destra (C 34). Circa 1/2.
2. Primo molare superiore destro (C 33).
   b) *Sus scrofa* LINNEO.
3. Femore destro (C 18). Norma anteriore.
4. Omerotro (C 16). Norma posterodistale.
5. Omero destro (C 15). Norma posterodistale.
6. Omero sinistro (C 38). Norma posterodistale.
7. Emimandibola sinistra (C 10).

TAV.2



TAV. 3 – a) *Aves gen. et sp. indet.*
1. Cubito destro (C 37).
2. Cubito destro (C 37).
3. Tibia (C 38).
4. Tibia (C 38).
5. Metatarso (C 39).
   b) *Ovis vel Capra*
6. Calcagno sinistro (C 32).
   c) Ceramica.
7 Frammento di vaso.

Tutte le figure sono in grandezza naturale tranne C 34 che è circa 1/2 dell'originale.


TAV.3
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Il Dissesto Idrogeologico

Vallario A.
Professore Ordinario di Geologia Applicata
Dipartimento di Scienze della Terra
Facoltà di Scienze, Università degli Studi «Federico II»

da una Conferenza alla Società dei Naturalisti in Napoli

Gli eventi naturali ed antropici succedutisi negli ultimi due secoli sono stati caratterizzati da un crescendo, rapido superamento di fasi storiche e sociali, in relazione alla progressiva affermazione e diffusione della società industriale, all'incremento della popolazione, all'ampliarsi di centri urbani, alla concentrazione delle aree industriali, al veloce progresso dello sviluppo tecnologico e all'utilizzo indiscriminato delle risorse naturali, trascurando nel contempo la qualità della vita, le condizioni di lavoro e gli effetti alterativi ed inquinanti che le attività antropiche avrebbero potuto determinare all'ambiente nell'immediato e nel futuro (Di Donna e Vallario, 1992, 1993a., 1993b; Vallario, 1992a).

In tale contesto il rapporto dell'uomo con l'ambiente naturale è stato improntato, prevalentemente, al principio dello sfruttamento. Posizione questa sviluppata dalle filosofie occidentali per le quali l'uomo è l'essere superiore in grado di sottomettere la natura. Va ricordato a tal proposito che anche l'insegnamento cristiano si pone nella stessa posizione come specificato in un versetto della Genesi che esorta l'uomo e la donna a crescere e moltiplicarsi ed ad usare la terra, rendendosela soggetta. (Dorst, 1988).

Solo negli ultimi decenni l'incalzante ripetersi di *catastrofi* e di disastrosi fenomeni alterativi dell'ambiente fisico (Catenacci, 1992), tra l'altro conseguenti al modelli socioeconomici prevalenti, ha determinato l'unanime ammissione che l'uomo ha avuto il ruolo di agente attivo nell'alterazione degli equilibri naturali. Infatti le attività antropiche, sempre più incisive ed irrispettose dell'ambiente fisico e delle risorse naturali, hanno agito come fattori della dinamica esogena al pari delle acque superficiali del vento e delle escursioni termiche, con l'aggravante che l'uomo agisce senza soluzione di continuità ritenendo, tra l'altro, di essere l'unico indiscusso padrone e beneficiario di tali beni, mentre avrebbe avuto interesse ad assumere il ruolo del rispettoso e raziocinante custode

di un patrimonio unico, comune a tutti gli esseri viventi, e non rinnovabile in tempi umani

Nel vasto panorama di equivoci voluti e determinati per perseguire lo sfruttamento intensivo ed estensivo delle potenzialità naturali ha svolto un ruolo determinante la mancanza di una visione complessiva delle problematiche ambientali e, soprattutto. di un disegno programmatico di indirizzo politico e gestionale che ha agevolato, quando non direttamente innescato, il progressivo depauperamento dell'ambiente fisico e delle risorse naturali (Ministero dell'Ambiente, 1992, Vallario, 1992b).

Ambiente fisico e potenzialità naturali

Ambiente è quanto comprende ed interagisce, mediante complesse relazioni funzionali dirette ed indirette con l'uomo, gli altri esseri viventi, il mondo inorganico, le condizioni geologiche, biologiche, fisiche e chimiche che costituiscono e, quindi caratterizzano un determinato spazio geografico. Tutti gli elementi costituenti l'ambiente risultano tra loro interdipendenti in vario modo e con diversa intensità, in tempi a scala geologica e umana, a seconda delle circostanze, dei processi e dei fenomeni considerati. Un ambiente rappresenta un sistema in cui al variare di condizioni interne e/o esterne possono mutare i rapporti tra le sue varie componenti; in tal modo alcune di esse, col procedere del tempo, possono perdere il carattere di prevalenza per essere sostituiti da altri che rappresentano l'effetto dei nuovi mutamenti. Il sistema ambiente non può, quindi, ritenersi statico ma in continua evoluzione dinamica (Vallario, 1992a).

La complessità degli elementi costitutivi e le loro ampie interrelazioni lasciano intendere che l'ambiente va considerato come una realtà unitaria nella sua struttura, nei suoi meccanismi e nei suoi equilibri dinamici e che l'uomo ha assunto, via via nel tempo. un ruolo sempre più determinante quale parte integrante del sistema.

Le tematiche inerenti all'ambiente che più direttamente interessano l'uomo devono avere come obiettivi gli strumenti per giungere ad una approfondita analisi dei fenomeni e la conoscenza delle potenzialità naturali, ciò per consentire una corretta pianificazione e gestione antropica del territorio.

Gli elementi di giudizio necessari a sviluppare un approccio culturale corretto delle problematiche ambientali derivano dalla conoscenza dei

fenomeni naturali. Questa, partendo dall'analisi delle componenti dell'ambiente, può consentire di giungere alla ricostruzione dei modelli morfoevolutivi e quindi alle previsioni sia sull'innesco di nuovi fenomeni alterativi che sulle reazioni agli stessi. Ciò equivale a prevedere e quindi programmare un'adeguata prevenzione ai conseguenti rischi per la vita e per le opere dell'uomo.

Le potenzialità naturali sono rappresentate oltre che dalle risorse rinnovabili e non,. quali le acque superficiali e sotterranee, l'aria, i combustibili fossili, i materiali naturali da costruzione, le fonti energetiche, le materie prime, anche dal paesaggio, dal suolo, dalla fauna, dalla flora, dai parchi naturali, dalle risorse alimentari e dalle caratteristiche fisiche dell'ambiente che in diversi casi hanno costituito presupposti indispensabili per lo sviluppo economico e sociale di alcune regioni (Vallario, 1991).

L'assetto e l'uso razionale del territorio dovrebbero scaturire dalle conoscenze acquisite, dalle caratteristiche fisiche dell'ambiente e dalla entità e tipologia delle potenzialità naturali disponibili in un certo ambito geografico.

Nell'approccio all'ambiente fisico sembra opportuno ricordare che la configurazione geomorfologica attuale di un certo sito rappresenta solo un istante a scala umana della sequenza che ha dato luogo nei tempi geologici al susseguirsi di processi e forme che hanno determinato l'evoluzione a cui è stata sottoposta quella porzione di superficie terrestre sotto l'azione degli agenti esogeni (acque meteoriche, escursioni termiche, ecc) ed endogeni (eventi tettonici, fenomeni vulcanici, sismi, ecc.). Aspetti questi che hanno costituito, da circa quaranta anni, argomenti di studio da parte di molti ricercatori italiani quali, ad esempio: Gisotti e Bruschi, 1990; Guida *et al.*, 1974, 1979, 1981; Ippolito, 1954, 1962, 1967, 1972a, 1972b, 1973; Vallario, 1973, 1992b, 1993a, 1993b).

Il rischio geologico

L'uomo vive ed opera, prevalentemente. in corrispondenza della porzione più superficiale della crosta terrestre; proprio in quella parte in cui avvengono, o si avvertono, gli effetti dei fenomeni di modellamento, di trasformazione, di alterazione, di adattamento o di reazione connessi, in modo diretto o indiretto. agli agenti della dinamica endogena ed esogena, (Bolt *et al.*, 1975, Vallario, 1992b).

L'insieme dei fenomeni geologici e dei loro effetti sulla superficie terrestre rappresenta quella che possiamo definire la *pericolosità geologica*, la porzione della superficie terrestre dove vivono ed operano comunità antropiche rappresenta, invece, la *potenziale vulnerabilità' antropica* di un dato territorio ai fenomeni geologici.

Il *rischio geologico* è la combinazione della *pericolosità geologica* e della *potenziale vulnerabilità antropica* di un territorio, espresso in termini di rapporto tra i prevedibili eventi di pericolosità geologica, la loro intensità e frequenza e le relative interferenze con le attività antropiche.

Tra gli eventi di *pericolosità geologica* devono rientrare sia i fenomeni naturali che quelli indotti dalle attività antropiche quali, ad esempio, i fenomeni vulcanici, i terremoti, le alluvioni, i maremoti, le erosioni intense della superficie terrestre, le frane, la subsidenza, il bradisismo, gli effetti delle grandi infrastrutture antropiche sull'evoluzione della superficie terrestre, l'inquinamento, ecc.

La *potenziale vulnerabilità antropica* può comprendere l'intensità e il tipo di urbanizzazione, l'uso del territorio, l'industrializzazione, la presenza di infrastrutture viarie, le opere di captazione di importanti sorgenti, opere di adduzione da liquidi o gas, opere di scarico di liquami, la presenza di bacini artificiali, ecc.

Da ciò deriva, quindi, che il *rischio geologico* è strettamente connesso alle attività antropiche e che può essere ridotto agendo non tanto sui fenomeni geologici, peraltro difficilmente controllabili, ma soprattutto, mediante un uso oculato e razionale del territorio da parte dell'uomo (Vallario 1991).

Aspetto particolarmente significativo per la definizione del *rischio geologico* in un determinato sito è la individuazione di tutti gli elementi geologici e delle cause che, di volta in volta, si combinano negativamente concorrendo a turbare gli equilibri, tali conoscenze dovranno applicarsi ai fattori di potenziale vulnerabilità per definire le strategie di intervento che costituiranno oggetto ed obiettivo della programmazione, della pianificazione e della gestione del territorio, per ben rispondere alle esigenze umane (Benedini e Gisotti, 1985; Greco, 1992; Vallario, 1992b).

Il dissesto idrogeologico

Tra i processi evolutivi di maggiore incisività e velocità che sconvolgono l'ambiente fisico sono da includere le frane, i fenomeni di intensa erosione e le alluvioni; fenomeni questi di primaria importanza per gli effetti catastrofici che possono produrre sulle opere, sulle attività e sulla

vita stessa degli uomini. Questi fenomeni che sconvolgono tanto profondamente l'ambiente fisico ed il territorio rientrano nelle fenomenologie del così detto dissesto idrogeologico, definizione questa che tende a mettere in rilievo che tali fenomeni alterativi hanno cause determinanti nelle acque superficiali e sotterranee.

Le catastrofi naturali che più frequentemente incombono sul territorio del nostro paese, sono da collegare a fenomeni di intensa erosione, a movimenti in massa o frane o ad alcuni particolari aspetti della dinamica fluviale (alluvioni) in quanto costituenti gli elementi più diffusi e maggiormente incisivi nell'evoluzione rapida dell'ambiente fisico, eventi questi che vengono comunemente indicati con la denominazione di *rischio idrogeologico* e comprendono quelle catastrofi, che derivano dalla frequente combinazione di irrazionali utilizzazioni antropiche dell'ambiente fisico e da, più o meno sfavorevoli, condizioni geologiche e morfologiche.

Sembra opportuno ribadire che l'evoluzione dell'ambiente fisico è condizionata prevalentemente dai processi di rapido modellamento conseguenti alla dinamica fluviale (alluvioni), alla dinamica dei versanti (fenomeni erosionali e frane) e alla dinamica dei litorali (variazioni delle linee di costa e dei fondali).

Si definisce alluvione l'insieme dei fenomeni legati all'alta velocità dell'acqua nella rete drenante superficiale, all'erosione, al trasporto e successivo deposito di ingenti quantità di materiale solido fine e grossolano, sia nell'alveo che nelle aree limitrofe, in occasione dello straripamento dei corsi d'acqua. Gli effetti di tali fenomeni sono disastrosi in quanto l' onda di piena che si propaga da monte a valle aumenta in portata e velocità. Con l'aumento della portata l'acqua, non più contenuta nell'alveo, può invadere le aree circostanti, con l'aumento della velocità essa si carica di detriti e incrementa ancor più la propria energia, provocando intensi fenomeni erosionali con successivo deposito del materiale eroso e trasportato.

Tali fenomeni stravolgono interi bacini imbriferi modificando sia le condizioni morfologiche precedenti e sia i modelli morfoevolutivi; i loro effetti disastrosi sono da collegare, oltre che a condizioni 0naturali, anche all'utilizzazione insensata dell'uomo di quelle parti pianeggianti, prospicienti gli alvei, che vengono invase dalle acque solo nei periodi di eccezionale piovosità.

Adeguate opere idrauliche di regimazione delle piene e di regolazione delle sponde e dei profili di fondo, la sistemazione dei bacini montani e il mantenimento di aree di rispetto per lo smaltimento delle piene sono gli

interventi che hanno consentito di ridurre notevolmente gli effetti distruttivi delle alluvioni.

Tra gli interventi antropici particolarmente incidenti sulla dinamica fluviale è indispensabile ricordare l'asportazione di ingenti quantità di materiali alluvionali dagli alvei dei corsi d'acqua; queste azioni hanno avuto incidenza via via crescente a partire dagli anni cinquanta, quando sotto la spinta del forte sviluppo economico sono iniziate le massicce realizzazioni di infrastrutture viarie e di innumerevoli costruzioni di ogni genere e tipo che hanno utilizzato i depositi alluvionali per realizzare rilevati stradali e per la confezione del calcestruzzo. Questo fenomeno, nel complesso rapporto tra i diversi elementi dell'ambiente fisico, ha provocato anche sostanziali modificazioni, a volte irreversibili, al regime dei litorali; inoltre, le asportazioni hanno generato l'abbassamento progressivo degli alvei con conseguenti crolli di manufatti, franamento delle difese di sponda e modificazioni, anche profonde, degli articolati sistemi erosione-trasporto-sedimentazione.

La dinamica dei versanti comprende i fenomeni di intensa erosione ed i movimenti in massa. I fenomeni erosionali sono legati quasi esclusivamente all'azione meccanica delle acque dilavanti che, per la forza di gravità, si spostano da monte a valle. I movimenti in massa o frane sono costituiti dalla caduta più o meno rapida o, comunque, dallo spostamento lento e differenziale di masse rocciose o di materiali sciolti, per gravità. In tal senso appare evidente che i fenomeni erosionali e le frane sono agenti modellatori particolarmente incisivi che non solo modificano localmente, ma intervengono in maniera determinante nell'evoluzione dell'ambiente fisico in quanto, alterando la geometria dei versanti e le forme del suolo, agevolano l'insorgere di nuovi meccanismi di trasformazione che si sovrappongono ai precedenti e, in alcuni casi, si sostituiscono ad essi. In molte zone del territorio nazionale le caratteristiche geologiche inducono all' instaurarsi diffuso di fenomeni di tal tipo che spesso raggiungono l'entità di vere e proprie *catastrofi naturali* (Vallario, 1992a e b).

L'agente principale della dinamica dei litorali è costituito dall'azione del moto ondoso; un ruolo del tutto subordinato spetta alle correnti marine. La tendenza evolutiva delle coste basse, quelle di maggiore interesse per le attività antropiche, oltre che dagli elementi naturali, dipende dagli interventi dell'uomo. I tratti di costa che si possono ritenere naturalmente stabili sono estremamente limitati in quanto è difficile che si possa realizzare l'equilibrio duraturo tra apporti, erosioni e trasporto dei materiali.

Altra azione antropica di alterazione degli equilibri è la costruzione di opere costiere quali moli, pennelli e scogliere che, modificando le modalità di trasporto del materiale lungo costa, provocano erosioni ed accumuli anomali rispetto agli schemi naturali.

Val la pena di ricordare che se non si ripristinano le condizioni naturali alterate dall'escavazione dei materiali dagli alvei dei corsi d'acqua, non si potranno raggiungere condizioni di equilibrio naturali. Unica alternativa concreta a tali situazioni è il ripascimento artificiale delle coste basse.

Il ripetersi di eventi catastrofici di ampiezza ed entità sempre maggiore e, in particolare, l'alluvione dell'Arno del 1966 suggerirono l'istituzione di una Commissione Interministeriale per avviare finalmente una ricognizione conoscitiva sul territorio nazionale.

Gli studi ed i rilevamenti effettuati misero in evidenza nel 1970 che era necessaria una spesa di 8.932 miliardi, da investire in un trentennio, per la difesa del suolo e la sistemazione idraulica sul territorio nazionale. In particolare erano previsti 5.300 miliardi per la difesa idraulica del suolo, 429 miliardi per la sistemazione di frane e la prevenzione di valanghe, 2.370 miliardi per la sistemazione idraulicoagraria e per il potenziamento silvo-pastorale e 824 miliardi per la difesa dei litorali (Commissione Interministeriale, 1970).

Da allora molto è cambiato soprattutto nello sfruttamento del territorio e delle risorse naturali, con conseguenze sempre più catastrofiche ed irreversibili. In ciò l'uomo ha avuto un ruolo prioritario e determinante ed è da ritenere l'agente modellatore che ha avuto le maggiori responsabilità rispetto agli eventi naturali.

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